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)

<table>
<thead>
<tr>
<th>Case number</th>
<th>Towna</th>
<th>Sex</th>
<th>Age in years</th>
<th>Immunisation status (MCV)</th>
<th>Onset of illness date (week number)</th>
<th>Sampling date (week number)</th>
<th>MV-specific antibodies</th>
<th>Laboratory confirmation and case classification</th>
<th>MV genotype and variant</th>
<th>GenBank accession number and WHO name</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV transmission chain, Rimini-Baden-Wuerttemberg-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.1 (index case)</td>
<td>A</td>
<td>M</td>
<td>16</td>
<td>Unvaccinated</td>
<td>15/06/11 (24)</td>
<td>21/06/11 (25)</td>
<td>+</td>
<td>+</td>
<td>12%</td>
<td>Yes, primary infection</td>
</tr>
<tr>
<td>1.2</td>
<td>A</td>
<td>M</td>
<td>19</td>
<td>Unvaccinated</td>
<td>27/06/11 (26)</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>1.3</td>
<td>A</td>
<td>F</td>
<td>14</td>
<td>Unvaccinated</td>
<td>27/06/11 (26)</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>MV transmission chain, Rimini-Baden-Wuerttemberg-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1 (index case)</td>
<td>A</td>
<td>M</td>
<td>18</td>
<td>Unknown</td>
<td>17/06/11 (24)</td>
<td>21/06/11 (25)</td>
<td>+</td>
<td>+</td>
<td>13%</td>
<td>Yes, primary infection</td>
</tr>
<tr>
<td>2.2</td>
<td>B</td>
<td>F</td>
<td>18</td>
<td>Unvaccinated</td>
<td>25/06/11 (25)</td>
<td>NA</td>
<td>+b</td>
<td>ND</td>
<td>ND</td>
<td>Yes, not classified</td>
</tr>
<tr>
<td>2.3</td>
<td>A</td>
<td>F</td>
<td>21</td>
<td>Unvaccinated</td>
<td>26/06/11 (25)</td>
<td>NA</td>
<td>+b</td>
<td>ND</td>
<td>ND</td>
<td>Yes, not classified</td>
</tr>
<tr>
<td>2.4</td>
<td>C</td>
<td>M</td>
<td>27</td>
<td>Unvaccinated</td>
<td>26/06/11 (25)</td>
<td>NA</td>
<td>+b</td>
<td>+b</td>
<td>ND</td>
<td>Yes, not classified</td>
</tr>
<tr>
<td>2.5</td>
<td>D</td>
<td>M</td>
<td>23</td>
<td>Unvaccinated</td>
<td>27/06/11 (26)</td>
<td>NA</td>
<td>+b</td>
<td>ND</td>
<td>ND</td>
<td>Yes, not classified</td>
</tr>
<tr>
<td>2.6</td>
<td>E</td>
<td>M</td>
<td>20</td>
<td>Unknown</td>
<td>27/06/11 (26)</td>
<td>07/07/11 (27)</td>
<td>+b</td>
<td>ND</td>
<td>ND</td>
<td>Yes, not classified</td>
</tr>
<tr>
<td>2.7</td>
<td>A</td>
<td>M</td>
<td>20</td>
<td>Unvaccinated</td>
<td>28/06/11 (26)</td>
<td>05/07/11 (27)</td>
<td>+</td>
<td>+</td>
<td>5%</td>
<td>Yes, primary infection</td>
</tr>
<tr>
<td>2.8</td>
<td>F</td>
<td>M</td>
<td>24</td>
<td>Unvaccinated</td>
<td>30/06/11 (26)</td>
<td>06/07/11 (27)</td>
<td>+</td>
<td>+</td>
<td>5%</td>
<td>Yes, primary infection</td>
</tr>
<tr>
<td>2.9</td>
<td>A</td>
<td>F</td>
<td>15</td>
<td>Unvaccinated</td>
<td>04/07/11 (27)</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>2.10</td>
<td>G</td>
<td>M</td>
<td>11</td>
<td>Unvaccinated</td>
<td>08/07/11 (27)</td>
<td>11/07/11 (28)</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>Yes, primary infection</td>
</tr>
<tr>
<td>MV transmission chain, Rimini-Slovenia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.1 (index case)</td>
<td>H</td>
<td>F</td>
<td>34</td>
<td>Unvaccinated</td>
<td>13/06/11 (24)</td>
<td>19/06/11 (24)</td>
<td>+</td>
<td>-/+</td>
<td>ND</td>
<td>Yes, primary infection</td>
</tr>
<tr>
<td>3.2</td>
<td>I</td>
<td>M</td>
<td>34</td>
<td>Unvaccinated</td>
<td>28/06/11 (26)</td>
<td>01/07/11 (26)</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>Yes, primary infection</td>
</tr>
<tr>
<td>3.3</td>
<td>J</td>
<td>M</td>
<td>33</td>
<td>Unvaccinated</td>
<td>30/06/11 (26)</td>
<td>03/07/11 (26)</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>Yes, primary infection</td>
</tr>
<tr>
<td>3.4</td>
<td>H</td>
<td>M</td>
<td>30</td>
<td>Vaccinated in 1981 and 1987</td>
<td>01/07/11 (26)</td>
<td>05/07/11 (27)</td>
<td>-</td>
<td>+</td>
<td>83%</td>
<td>Yes, SVF</td>
</tr>
</tbody>
</table>

+: 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>
<thead>
<tr>
<th>Case number</th>
<th>Towna</th>
<th>Sex</th>
<th>Age in years</th>
<th>Immunisation status (MCV)</th>
<th>Onset of illness date (week number)</th>
<th>Sampling date (week number)</th>
<th>MV-specific antibodies</th>
<th>Laboratory confirmation and case classification</th>
<th>MV genotype and variant</th>
<th>GenBank accession number and WHO name</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.6</td>
<td>H</td>
<td>F</td>
<td>28</td>
<td>Vaccinated in 1984</td>
<td>29/06/11 (26)</td>
<td>01/07/11 (26)</td>
<td>-</td>
<td>Yes, VF not classifiable</td>
<td>D8-V</td>
<td>–</td>
</tr>
<tr>
<td>3.7</td>
<td>H</td>
<td>F</td>
<td>42</td>
<td>Vaccinated in 1969</td>
<td>02/07/11 (26)</td>
<td>04/07/11 (27)</td>
<td>+</td>
<td>Yes, SVF</td>
<td>D8-V</td>
<td>–</td>
</tr>
<tr>
<td>3.8</td>
<td>K</td>
<td>M</td>
<td>36</td>
<td>Vaccinated in 1975 and 1981</td>
<td>02/07/11 (26)</td>
<td>05/07/11 (27)</td>
<td>-</td>
<td>Yes, SVF</td>
<td>D8-V</td>
<td>KJ411831 MVs/Novo Mesto.SVN/27.11</td>
</tr>
<tr>
<td>3.9</td>
<td>H</td>
<td>F</td>
<td>47</td>
<td>Unvaccinated</td>
<td>02/07/11 (26)</td>
<td>06/07/11 (27)</td>
<td>+/-</td>
<td>Yes, primary infection</td>
<td>D8-V</td>
<td>–</td>
</tr>
<tr>
<td>3.10</td>
<td>H</td>
<td>M</td>
<td>35</td>
<td>Vaccinated in 1977 and 1983</td>
<td>04/07/11 (27)</td>
<td>04/07/11 (w. 27)</td>
<td>-</td>
<td>Yes, SVF</td>
<td>D8-V</td>
<td>–</td>
</tr>
<tr>
<td>3.11</td>
<td>H</td>
<td>M</td>
<td>33</td>
<td>Vaccinated in 1978 and 1984</td>
<td>NA</td>
<td>04/07/11 (27)</td>
<td>-</td>
<td>Yes, SVF</td>
<td>D8-V</td>
<td>–</td>
</tr>
<tr>
<td>3.12</td>
<td>H</td>
<td>M</td>
<td>32</td>
<td>Vaccinated in 1979 and 1985 (documented)</td>
<td>NA</td>
<td>05/07/11 (27)</td>
<td>+</td>
<td>Yes, probable SVF</td>
<td>D8-V</td>
<td>–</td>
</tr>
<tr>
<td>3.13</td>
<td>H</td>
<td>F</td>
<td>33</td>
<td>Vaccinated in 1978 and 1984 (documented)</td>
<td>13/07/11 (28)</td>
<td>15/07/11 (28)</td>
<td>+</td>
<td>Yes, SVF</td>
<td>D8-V</td>
<td>–</td>
</tr>
<tr>
<td>4d</td>
<td>H</td>
<td>F</td>
<td>44</td>
<td>Vaccinated in 1973</td>
<td>29/06/11 (26)</td>
<td>04/07/11 (27)</td>
<td>+</td>
<td>Yes, SVF</td>
<td>D8-V</td>
<td>–</td>
</tr>
<tr>
<td>5d</td>
<td>H</td>
<td>M</td>
<td>35</td>
<td>Unvaccinated</td>
<td>11/07/11 (28)</td>
<td>16/07/11 (28)</td>
<td>-</td>
<td>Yes, primary infection</td>
<td>D8-V</td>
<td>KJ411830 MVs/Ljubljana.SVN/28.11</td>
</tr>
</tbody>
</table>

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

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.

Investigation performed by external laboratory.

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

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, indicating spread of the imported virus in Slovenia. 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.)

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.)
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
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, 195% 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-meatles 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 Kurzenndörfer, Anne Wölbert, 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
2. Mankertz A, Mulders MM, Shulga S, Kremer JR, Brown KE, Santibanez S, 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.

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
2. Mankertz A, Mulders MM, Shulga S, Kremer JR, Brown KE, Santibanez S, 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.

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.

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.


