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Review

Molecular surveillance of measles and rubella in the WHO European Region: new challenges in the elimination phase

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ABSTRACT

Background: The WHO European Region (EUR) has adopted the goal of eliminating measles and rubella but individual countries perform differently in achieving this goal. Measles virus spread across the EUR by mobile groups has recently led to large outbreaks in the insufficiently vaccinated resident population. As an instrument for monitoring the elimination process and verifying the interruption of endemic virus transmission, molecular surveillance has to provide valid and representative data. Irrespective of the country's specific situation, it is required to ensure the functionality of the laboratory surveillance that is supported by the WHO Global Measles and Rubella Laboratory Network.

Aims: To investigate whether the molecular surveillance in the EUR is adequate for the challenges in the elimination phase, we addressed the quality assurance of molecular data, the continuity and intensity of molecular monitoring, and the analysis of transmission chains.

Sources: Published articles, the molecular External Quality Assessment Programme of the WHO, the Centralized Information System for Infectious Diseases of the WHO EUR and the WHO Measles and Rubella Nucleotide Surveillance databases served as information sources.

Content: Molecular proficiency testing conducted by the WHO in 2016 has shown that the expertise for measles and rubella virus genotyping exists in all parts of the EUR. The analysis of surveillance data reported nationally to the WHO in 2013–2016 has revealed some countries with outbreaks but not sufficiently representative molecular data. Long-lasting supranational MV transmission chains were identified.

Implications: A more systematic molecular monitoring and recording of the transmission pattern for the whole EUR could help to create a meaningful picture of the elimination process. **S. Santibanez, Clin Microbiol Infect 2017;23:516**

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Introduction

All six regions of the WHO have established the goal of measles elimination, and three regions including the European Region (EUR) have set an elimination goal for rubella and the congenital rubella syndrome [1,2].

As part of the measles and rubella surveillance system, molecular surveillance is an important tool for monitoring the circulation of wild-type viruses in a region over time. Information on genotype and sequence variants of circulating viruses correlated with the epidemiological case data can help to identify the potential sources of virus importation and recognize long-lasting virus transmission chains. Endemic transmission is defined as continuous transmission of an indigenous or imported measles virus (MV) or rubella virus (RV) for a period of 12 months or more in a defined geographical area. The diseases are eliminated when the absence of endemic cases for a period of at least 12 months has been documented by a well-performing surveillance system [2]. Molecular surveillance is therefore challenged to monitor the elimination process, document the progress, and finally to support the evidence that interruption of transmission of endemic viruses is reached and can be maintained.

The MV genome is a non-segmented, negative-sense RNA that contains six genes coding for six structural proteins (N, P, M, F, H and L) plus two non-structural proteins (C and V) [3]. Although MV is considered a monotypic virus, genetic and antigenic variations have been detected in wild-type viruses [4]. According to the standardized WHO nomenclature using the nucleotide (nt) sequences of the variable genes N and H, wild-type MV are divided into 24 genotypes. The highly variable 450-nt sequence encoding the C-terminal part of the N protein (N-450 region) is used to differentiate detected MV for the purpose of surveillance [5]. A web-accessible and quality-controlled measles nucleotide surveillance database (MeaNS, http://www.who-measles.org/) was developed by Public Health England for the WHO Global Measles and Rubella Laboratory Network (GMRLN) [6]. MeaNS collects sequence data of the N-450 region, as well as the complete coding regions of the six individual genes and the whole genome plus the relevant case-specific epidemiological information. Various bioinformatics tools provided by MeaNS can be used for tracking transmission pathways of circulating sequence variants at regional and global levels. The nomination of frequently submitted sequence variants as 'named strains' facilitates the communication within the network and helps to recognize epidemiological relevant and potentially endemic MV sequence variants. MeaNS enables the user to track the sequence diversity of wild-type MV and monitor the elimination of MV strains and genotypes in an increasingly vaccinated population [7].

The RV genome contains two open reading frames, with the 5′ one coding for the two non-structural proteins P150 and P90 and the 3′ one coding for the three structural proteins C, E1 and E2 [8]. Based on 739 nt of the E1 gene, 12 recognized and one provisional genotype were defined, with 2B, 1E, 1G and 1J being the most frequently reported [9]. Rubella virus sequence data are stored in the Public Health England developed and quality-controlled database RubeNS (http://www.who-rubella.org/) that, similarly to MeaNS, enables the user to find identical sequences and genotype new RV sequence variants. RubeNS can be used to track the RV sequence diversity and monitor the elimination of RV strains.

Laboratory-based surveillance of measles and rubella is coordinated by the WHO GMRLN. In 2015, surveillance in 191 countries was supported by 703 GMRLN laboratories based in 165 countries [1]. The GMRLN provides comprehensive technical support and opportunities for education and training, enables sharing of expertise and data and has established programmes for quality assurance. Since the start of molecular investigation of MV and RV in the EUR in the early 1990s, there has been an ongoing shift from analysis of randomly collected samples and data in a few countries to an increasingly systematic, area-wide and continuous molecular surveillance with reporting to the WHO GMRLN on a regular basis. The national reference laboratories in GMRLN are encouraged to submit their data as soon as they become available to MeaNS and RubeNS. The data are therefore accessible to all contributing users in an almost real-time modus and help to recognize and analyse virus transmission chains in a global context.

As an instrument for verifying the interruption of endemic MV and RV transmission in the region, molecular surveillance has to provide valid and representative data that should be consistent with information provided by the other components of the surveillance system [2]. The present study investigates whether the performance of molecular surveillance in the EUR is adequate to the challenges in the elimination process. We address the quality assurance of molecular data, the continuity and intensity of nationally conducted molecular monitoring, and the analysis of supranational and long-lasting transmission chains.

Quality of molecular data

Particularly in the elimination phase, it is of increasing importance to ensure a high quality of the laboratory data used for molecular surveillance. Correct determination of the sequence of detected viral RNA is required for distinguishing between circulating virus variants. A molecular External Quality Assessment Programme has been developed by the CDC and the WHO with the goal to assess the performance of the GMRLN laboratories that routinely conduct virus genome detection and characterization [1,10]. The programme should serve to uncover problems in the methodology and the evaluation of laboratory-generated raw data in order to provide support, education and training. It was recently rolled out to the WHO EUR national measles and rubella laboratories in collaboration with INSTAND e.V. The first molecular proficiency testing exercise with the participation of about two-thirds of the 53 EUR countries has shown that MV and RV genome detection is established and accurately conducted in most of the EUR countries. In contrast, error-free sequencing was seen for measles in less than half of the EUR countries and for rubella in less than a quarter. Errors in measles sequencing were mainly caused by misinterpretation of laboratory raw data, whereas in the case of rubella the laboratory method used was not adequate. Overall, the capability of molecular detection of MV and RV exists EUR-wide and the expertise for genotyping is available in all parts of the region.

Continuity and intensity of molecular surveillance

To identify potential gaps in molecular surveillance that could lead to an incomplete recording of the MV and RV transmission patterns, and consequently to a misinterpretation of the situation in the country or the whole EUR, we analysed whether the reporting of the cases coincided in a timely manner with the submission of sequence data to MeaNS and RubeNS. For that purpose, the Centralized Information System for Infectious Diseases (CISID) of the WHO Regional Office for Europe as well as the MeaNS and RubeNS databases served as data sources [11]. The total number of measles and rubella cases reported monthly by the countries to CISID comprises laboratory confirmed, epidemiologically linked and clinically compatible cases. Countries that reported to CISID high numbers of cases ($\geq$1000 measles cases or $\geq$100 rubella cases) for at least 1 year of the investigation period 2013–2016 were

selected for analysis. For the selected countries only the years with ≥100 reported measles cases or ≥10 reported rubella cases were considered. Based on monthly data, we examined whether the sequence records in MeaNS and RubeNS reflect the reported cases during these periods.

Based on the defined algorithm, 12 countries were selected for analysis of measles data. MeaNS research revealed that five countries conducted genotyping continuously or with few and short temporal gaps whereas seven countries showed gaps of ≥3 months (Table 1). For six of them, sequence data are missing over several months in which very high case numbers (≥100 cases monthly) were reported to CISID. This demonstrates that the molecular data does not completely represent the actual MV transmission chains in the EUR. The ratio of the number of cases with genotype information deposited in MeaNS to the number of cases reported to CISID differs considerably between the selected countries and there is consequently a risk of incomplete recording of circulating MV sequence variants in some areas of EUR. Overall, the discontinuity and low intensity of MV molecular monitoring, particularly in countries that recently experienced large outbreaks [12–14], imply the need to shift towards a more systematically conducted molecular surveillance in the EUR.

Table 1
Temporal distribution of epidemiological versus molecular surveillance data for measles

Country	*Number of laboratory confirmed, epidemiologically linked and clinically compatible measles cases reported to CISID*																							
	Number of nucleotide sequence records in MeaNS																							
	2013												2014											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Bosnia and Herzegovina													1	*38*	*144*	*371*	*575*	*573*	*502*	*0*	*0*	*0*	*0*	*0*
														3	**4**	**2**		**7**	**2**			**1**	**1**	
Georgia	*4*	*54*	*593*	*1987*	*2446*	*1522*	*662*	*156*	*142*	*65*	*93*	*144*	*200*	*244*	*484*	*752*	*713*	*445*	*181*	*70*	*21*	*13*	*29*	*38*
		7	**30**	**7**			**1**						**2**	**9**	**18**	**14**								
Germany	*10*	*16*	*60*	*177*	*533*	*355*	*258*	*142*	*108*	*60*	*39*	*24*	*16*	*31*	*45*	*34*	*31*	*24*	*13*	*37*	*26*	*29*	*43*	*196*
	1		**4**	**9**	**15**	**11**	**14**	**10**	**7**	**4**	**5**	**1**	**1**	**6**	**2**	**5**	**5**	**2**	**3**	**8**	**6**	**9**	**1**	**7**
Italy	*103*	*337*	*314*	*241*	*387*	*429*	*211*	*81*	*58*	*43*	*270*	*458*	*1485*	*682*	*904*	*476*	*235*	*136*	*111*	*86*	*71*	*65*	*53*	*48*
	3	**9**	**15**	**5**	**13**	**9**	**10**	**7**	**2**	**3**	**5**		**8**	**20**	**33**	**17**	**12**	**9**	**14**	**10**	**5**	**13**	**8**	**5**
Kazakhstan													*12*	*25*	*47*	*56*	*27*	*43*	*12*	*13*	*11*	*27*	*22*	*22*
														2	**2**	**3**								
Kyrgyzstan													*0*	*0*	*0*	*0*	*11*	*36*	*8*	*20*	*9*	*4*	*28*	*203*
																		1						**3**
Netherlands	*1*	*4*	*10*	*5*	*18*	*295*	*700*	*407*	*444*	*473*	*177*	*106*	*46*	*24*	*42*	*21*	*5*	*1*	*1*	*2*	*2*	*0*	*0*	*0*
	1	**3**	**6**	**2**	**2**									**7**	**10**	**7**					**1**			
Romania	*227*	*219*	*182*	*146*	*80*	*127*	*83*	*27*	*11*	*23*	*21*	*12*												
	1	**1**	**9**	**4**	**1**	**2**	**1**	**1**			**3**													
Russian Federation	*22*	*40*	*75*	*157*	*266*	*125*	*63*	*112*	*125*	*242*	*556*	*718*	*1171*	*733*	*540*	*357*	*183*	*63*	*74*	*31*	*51*	*19*	*15*	*27*
	5	**7**	**22**	**43**	**37**	**13**	**13**	**35**	**26**	**29**	**40**	**63**	**83**	**72**	**34**	**39**	**24**	**26**	**25**	**3**	**3**	**5**	**9**	**11**
Turkey	*586*	*1114*	*1466*	*1313*	*1267*	*860*	*378*	*89*	*52*	*73*	*102*	*115*	*95*	*160*	*118*	*77*	*49*	*40*	*22*	*8*	*0*	*1*	*0*	*2*
	12	**6**	**3**	**11**	**9**				**1**	**4**			**2**	**5**	**3**	**1**	**4**	**2**		**1**				
Ukraine	*419*	*386*	*241*	*252*	*266*	*257*	*138*	*47*	*149*	*401*	*535*	*217*	*394*	*261*	*322*	*312*	*384*	*287*	*151*	*37*	*52*	*29*	*60*	*37*
	1	**2**	**5**	**1**	**3**	**2**	**2**			**1**				**2**		**2**		**1**	**3**				**3**	**1**
United Kingdom of Great Britain and Northern Ireland	*275*	*267*	*359*	*484*	*234*	*125*	*77*	*22*	*15*	*28*	*13*	*11*	*39*	*30*	*27*	*16*	*3*	*4*	*21*	*1*	*1*	*2*	*2*	*1*
	205	**121**	**47**	**103**	**51**	**36**	**57**	**18**	**14**	**19**	**11**	**6**	**29**	**20**	**23**	**11**	**3**	**2**	**11**	**2**	**1**	**1**		**3**

Case numbers reported to the Centralized Information System for Infectious Diseases (CISID) of the WHO Regional Office for Europe (data as 10 July 2017, marked in *italics*) and numbers of nucleotide sequence records in the database MeaNS (data as 11 June 2017, marked in *bold*) are compared. Only countries that reported ≥1000 cases for at least 1 year of the period 2013–2016 were included, and only years with ≥100 reported cases by country were considered.

Table 2
Temporal distribution of epidemiological versus molecular surveillance data for rubella

Country	*Number of laboratory confirmed, epidemiologically linked and clinically compatible rubella cases reported to CISID*																							
	Number of nucleotide sequence records in RubeNS																							
	2013												2014											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Georgia	*2*	*3*	*32*	*45*	*41*	*27*	*17*	*6*	*8*	*13*	*13*	*17*	*6*	*19*	*11*	*24*	*29*	*15*	*6*	*8*	*0*	*10*	*10*	*12*
Germany													*12*	*19*	*20*	*13*	*23*	*12*	*20*	*7*	*8*	*7*	*8*	*2*
Kazakhstan	*0*	*1*	*0*	*0*	*0*	*20*	*9*	*6*	*0*	*0*	*0*	*0*	*2*	*26*	*42*	*29*	*25*	*13*	*12*	*3*	*0*	*0*	*0*	*0*
Kyrgyzstan	*1*	*0*	*0*	*1*	*2*	*6*	*1*	*0*	*0*	*0*	*0*	*1*	*0*	*3*	*0*	*2*	*6*	*2*	*7*	*6*	*0*	*0*	*2*	*0*
Poland	*1833*	*2690*	*5918*	*10856*	*8466*	*4114*	*1877*	*690*	*569*	*606*	*481*	*485*	*770*	*672*	*913*	*837*	*822*	*493*	*410*	*157*	*159*	*201*	*226*	*239*
Romania	*28*	*46*	*26*	*40*	*38*	*36*	*20*	*28*	*16*	*6*	*0*	*6*	*20*	*0*	*18*	*6*	*4*	*2*	*2*	*4*	*0*	*0*	*2*	*2*
Russian Federation	*53*	*41*	*39*	*19*	*17*	*11*	*2*	*5*	*1*	*2*	*3*	*234*	*14*	*11*	*12*	*9*	*6*	*4*	*0*	*0*	*0*	*0*	*0*	*5*
	1	**2**	**2**										**1**		**1**	**3**			**1**					**1**

Case numbers reported to the Centralized Information System for Infectious Diseases (CISID) of the WHO Regional Office for Europe (data as 10 July 2017, marked in *italics*) and numbers of nucleotide sequence records in the database RubeNS (data as 11 June 2017, marked in *bold*) are compared. Only countries that reported ≥100 cases for at least 1 year of the period 2013–2016 were included, and only years with ≥10 reported cases by country were considered.

Sequence data for RV were submitted to RubeNS by only two of the seven selected countries and genotype information is available for only a very few cases (Table 2). One country reported a total of >40 000 cases in the analysed period to CISID, but there is no single case with genotype information [12–14]. Overall, the very limited availability of molecular data makes it impossible to assess to what extent RV transmission occurs in the EUR.

Supranational MV transmission chains

The beginning of molecular surveillance in the EUR in the early 1990s was characterized by detection of only two MV genotypes (C2 and D6) that were widespread throughout the region and therefore referred to as the indigenous European genotypes [15–18]. The disappearance of the C2 and D6 genotypes observed in the individual countries of the EUR between 2000 and 2007 coincided with the beginning of the period of successive cycles of initiation and termination of long-lasting transmission by various imported genotypes, as demonstrated by the example of Germany (Fig. 1) [19–21]. Genotypes B3, D4, D5, D7 and D8 originated from endemic areas overseas and initiated large outbreaks in the EUR [19–33]. There is a sustained risk of virus importation into the EUR by refugees and through unprotected persons travelling to endemic areas. The mobility of various risk groups with a high proportion of unprotected individuals across the EUR and the immunity gaps that still exist in the resident population of some countries favour su-

Country	*Number of laboratory confirmed, epidemiologically linked and clinically compatible measles cases reported to CISID*																							
	Number of nucleotide sequence records in MeaNS																							
	2015												2016											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Bosnia and Herzegovina	*455*	*515*	*433*	*354*	*283*	*113*	*53*	*8*	*4*	*1*	*15*	*4*												
	3	**19**																						
Georgia	*44*	*81*	*61*	*55*	*107*	*65*	*12*	*3*	*1*	*0*	*0*	*1*												
			1	**1**		**1**						**2**												
Germany	*432*	*443*	*608*	*442*	*234*	*109*	*64*	*18*	*15*	*12*	*2*	*4*	*7*	*7*	*15*	*33*	*60*	*51*	*44*	*29*	*24*	*16*	*31*	*11*
	33	**32**	**52**	**44**	**31**	**14**	**18**	**7**	**3**	**4**			**3**	**2**	**3**	**6**	**14**	**23**	**8**	**12**	**4**	**6**	**7**	**6**
Italy	*12*	*17*	*14*	*32*	*33*	*11*	*20*	*16*	*15*	*13*	*33*	*45*	*78*	*82*	*73*	*77*	*83*	*85*	*45*	*34*	*54*	*80*	*85*	*88*
	2	**4**	**4**	**5**	**5**		**3**	**5**	**4**	**4**	**6**	**7**	**24**	**24**	**25**	**31**	**22**	**13**	12	18	26	16	17	11
Kazakhstan	*220*	*485*	*426*	*515*	*347*	*232*	*58*	*24*	*7*	*7*	*6*	*13*	*6*	*14*	*43*	*13*	*19*	*0*	*11*	*0*	*0*	*0*	*0*	*0*
	6	**4**	**2**	**3**	**2**			**1**						**2**	**2**		**4**							
Kyrgyzstan	*2070*	*4359*	*4188*	*4435*	*2154*	*526*	*47*	*0*	*0*	*0*	*0*	*0*												
	7	**2**																						
Netherlands																								
Romania													*6*	*41*	*62*	*67*	*78*	*117*	*114*	*182*	219	426	614	506
													1	1	2	1	3		1	3	2	8	4	3
Russian Federation	*101*	*116*	*73*	*120*	*138*	*57*	*22*	*7*	*1*	*0*	*0*	*0*												
	7	**45**	**31**	**47**	**43**	**28**	**21**	**4**	**1**	**2**	**1**	**1**												
Turkey	*7*	*8*	*21*	*43*	*92*	*36*	*39*	*51*	*36*	*8*	*1*	*0*												
		4	**1**	**1**	**11**	**3**	**1**	**6**																
Ukraine	*25*	*22*	*7*	*14*	*14*	*7*	*6*	*38*	*2*	*3*	*2*	*1*												
United Kingdom of Great Britain and Northern Ireland													*4*	*22*	*44*	*56*	*48*	*76*	*132*	*108*	*29*	*37*	*14*	*1*
													3	**12**	**31**	**52**	**28**	**49**	**86**	**75**	**34**	**30**	**15**	**1**

Country	*Number of laboratory confirmed, epidemiologically linked and clinically compatible rubella cases reported to CISID*																							
	Number of nucleotide sequence records in RubeNS																							
	2015												2016											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Georgia	*8*	*10*	*11*	*11*	*16*	*20*	*7*	*5*	*1*	*1*	*4*	*6*	*2*	0	0	1	3	3	1	0	0	1	0	1
Germany	*11*	*7*	*7*	*15*	*9*	*8*	*10*	*4*	*5*	*3*	*5*	*5*	*6*	6	4	19	13	10	16	3	3	5	7	4
				1																				
Kazakhstan																								
Kyrgyzstan	*2*	*16*	*33*	*25*	*13*	*4*	*7*	*0*	*0*	*0*	*0*	*0*												
Poland	*216*	*199*	*256*	*231*	*183*	*165*	*142*	*114*	*92*	*149*	*140*	*142*	*142*	*138*	*111*	*151*	*110*	*116*	*54*	*53*	*48*	*81*	*64*	*76*
Romania													*0*	*0*	*1*	*1*	*2*	*1*	*0*	*3*	*0*	*1*	*2*	*2*
Russian Federation	*3*	*5*	*3*	*0*	*1*	*0*	*0*	*1*	*0*	*0*	*0*	*1*	*6*	*8*	*11*	*6*	*1*	*1*	*0*	*0*	*3*	*0*	*0*	*1*
			2									**1**	**1**		**3**	**1**		**1**			**1**			

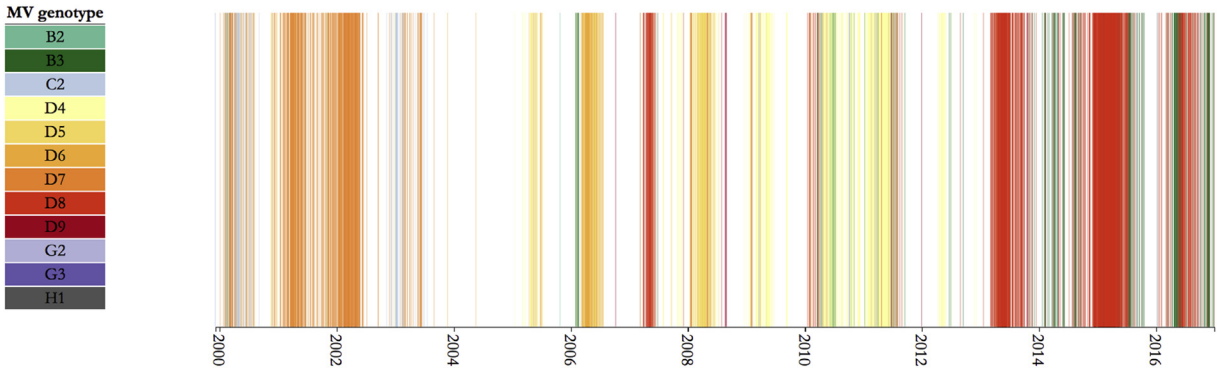

Fig. 1. Time series of MV genotypes in Germany. Each case is depicted by a vertical line. Color differentiates genotypes. In total 2665 cases are depicted for the period Dec. 1999 to Dec. 2016.

pranational transmission chains that are associated with large outbreaks [26,34,35]. Hence, a supranational analysis of the transmission patterns seems to be adequate for assessing the situation in the whole EUR. To determine the epidemiologically relevant MV sequence variants in the region, we have summarized the data recorded in the MeaNS for the 'named strains' of the predominant genotypes in 2013–2016, D8 and B3, and analysed their phylogeny (N-450 region).

The MV genotype B3 is endemic on the African continent [6] and was frequently imported into EUR countries [22,23,36]. Spread to the Philippines led to a large outbreak during 2013–2014 [37]. In the EUR, several outbreaks associated with genotype B3 were reported for the period 2013–2016 [12–14,38,39]. Most of the 'named strains' nominated for this genotype were circulating in EUR countries (Fig. 2). The named strain MVi/Harare.ZWE/38.09 was repeatedly imported from the Philippines outbreak and widely distributed in all parts of the EUR (data in MeaNS). Endemic transmission in the context of the whole EUR can be assumed for the named strains MVs/Niger.NGA/8.13 and MVs/Dublin.IRL/8.16. Both strains were detected mainly in the EUR, where they continue to circulate (data in MeaNS, Fig. 2).

The MV genotype D8 is endemic on the Indian subcontinent [6,40] and has been frequently imported into the EUR, where it was widely distributed in 2013–2016 and showed a high genetic diversity [12–14]. Most of the 'named strains' nominated for genotype D8 were highly prevalent in the EUR; endemic transmission

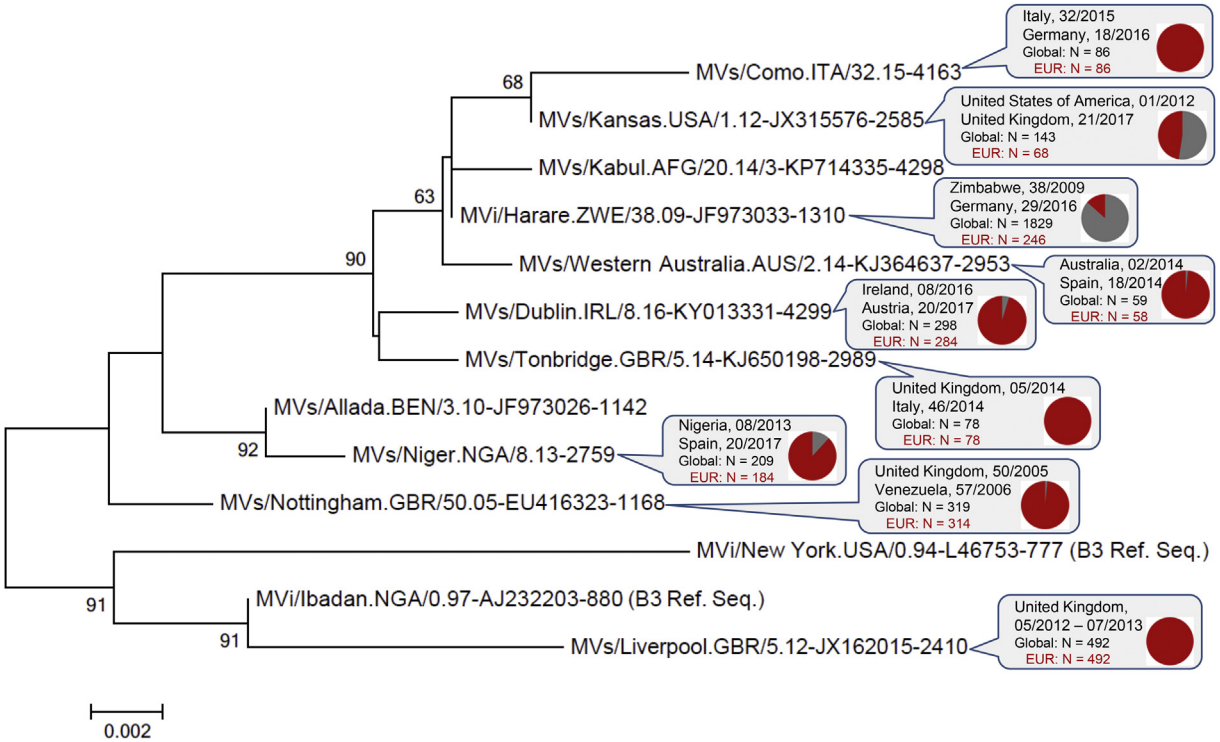

Fig. 2. Phylogenetic relationship between the epidemiologically relevant sequence variants ('named strains' in MeaNS) of MV genotype B3. The WHO name, the GenBank accession number (if available) and the 'distinct sequence identifier' used in MeaNS are given for each sequence variant. The most prevalent variants in the EUR (≥50 records in MeaNS) are annotated with information on location and dates of first and last detection globally and the number of records submitted globally and by European Region countries (data as 21 June 2017). The phylogenetic analysis is based on the 450-nt sequence encoding the C-terminus of the measles virus N-protein. The tree was constructed using the Neighbor-Joining algorithm and the p-distance method included in MEGA7 [46]. Only bootstrap values (1000 replicates) of at least 60 are shown. The scale bar indicates deviation of 2 nt per 1000-nt sequence.

can be assumed for the named strains MVs/Frankfurt Main.DEU/17.11, MVs/Republic of Komi.RUS/35.13 and MVs/Rostov on Don.RUS/47.13/2 (data in MeaNS, Fig. 3). The topology of their sequences in the phylogenetic tree suggests that all three strains belong to the same genetic lineage (Fig. 3). This lineage was detected mainly in the EUR where virus spread by travellers has led to large outbreaks in several parts of the region [32,33,35]. A genetic ancestry can also be assumed between other named strains of genotype D8 with a high prevalence in the EUR (i.e. MVs/Swansea.GBR/4.13 might have descended from MVs/Taunton.GBR/27.12, or MVs/Chui.KGZ/53.14 from MVi/Villupuram.IND/03.07) (Fig. 3).

Discussion and conclusions

Molecular surveillance of MV and RV monitors the elimination process in concert with the other components of the surveillance system and is therefore challenged to document the progress towards elimination and finally to support the evidence for interruption of endemic virus transmission. A well-performing molecular surveillance system is able to acquire adequate, representative and continuously collected clinical samples that are documented by case-based clinical and epidemiological data. In the elimination phase, correct detection and genetic identification of viruses have to be ensured by a quality-controlled methodology and it is crucial that the molecular data are fully integrated with epidemiological case-based surveillance [2].

The progress reached towards elimination differs between the individual EUR countries. Some countries have recently experienced large or moderate outbreaks whereas others have reported no or only very few, mostly imported, cases for several years [12–14]. Irrespective of the country's specific situation, it is required to ensure the functionality of the national molecular surveillance system and its preparedness to act rapidly. The molecular External Quality Assessment Programme of the WHO enables participants to assess their proficiency and also facilitates comparison of methods and sharing of experience [1]. It provides an opportunity of regular training, in particular for laboratories in countries nearing elimination investigating only very few cases in their routine work. All laboratories that provide molecular-based information for surveillance should take part in the programme with the aim of achieving coverage of the whole EUR by a quality-controlled molecular surveillance.

This study has shown that some countries reported high numbers of measles cases in 2013–2016, but the molecular data were not continuously collected and are therefore not sufficiently representative. For countries that do not follow the guidelines for measles and rubella outbreak investigation in the EUR, it is not possible to distinguish between a single nationwide outbreak caused by one imported virus variant and the simultaneous occurrence of various outbreaks caused by different independently imported variants [41]. Likewise, it cannot be distinguished between a single long-lasting outbreak and occurrence of distinct successive or timely overlapping outbreaks, with the consequence that the epidemiological situation cannot be assessed. A shift from the currently sporadic to a systematically conducted molecular surveillance is therefore necessary in some countries to avoid transmission chains remaining unrecognized or underestimated in their extension. This development could be facilitated by a regular and close collaboration between epidemiologists, the local public health institutions and the laboratory.

For rubella, molecular data for the period 2013–2016 were available for only a few cases from the EUR, although several countries reported that cases occurred almost continuously or over periods of several months. The molecular proficiency testing has

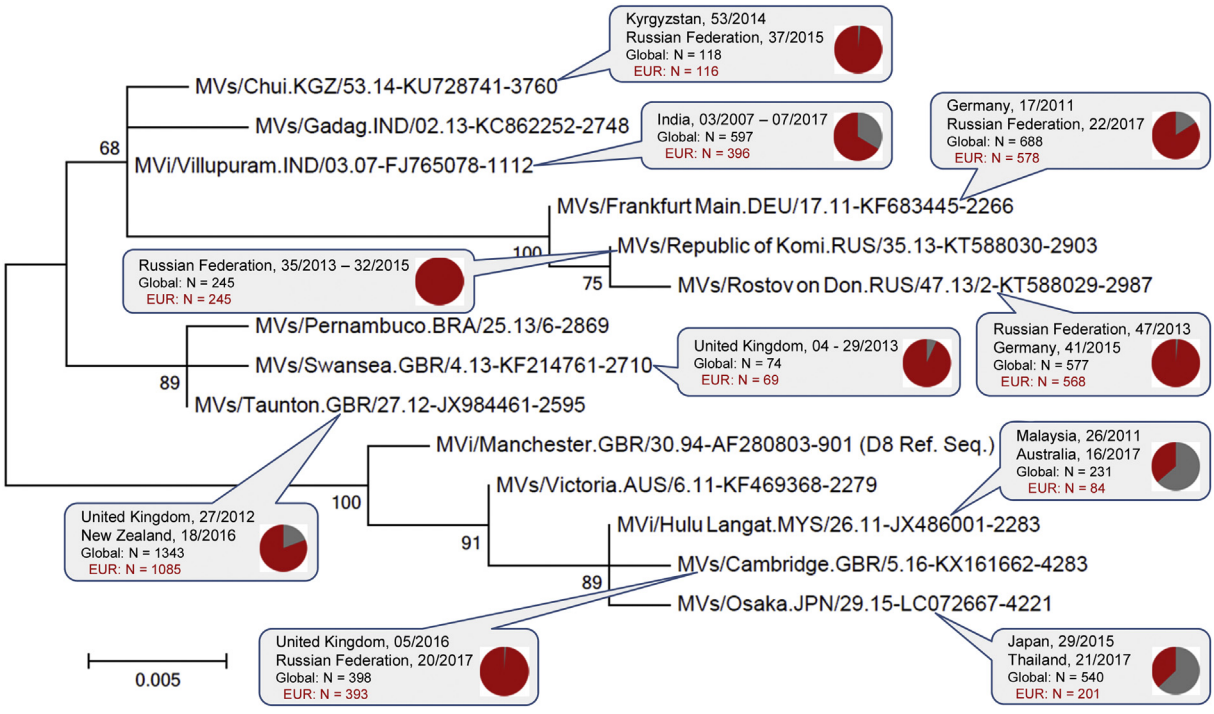

Fig. 3. Phylogenetic relationship between the epidemiologically relevant sequence variants ('named strains' in MeaNS) of MV genotype D8. The WHO name, the GenBank accession number (if available) and the 'distinct sequence identifier' used in MeaNS are given for each sequence variant. The most prevalent variants in the EUR (≥50 records in MeaNS) are annotated with information on location and dates of first and last detection globally and the number of records submitted globally and by European Region countries (data as 21 June 2017). The phylogenetic analysis is based on the 450-nt sequence encoding the C-terminus of the measles virus N-protein. The tree was constructed using the neighbour-joining algorithm and the p-distance method included in MEGA7 [46]. Only bootstrap values (1000 replicates) of at least 60 are shown. The scale bar indicates deviation of 5 nt per 1000-nt sequence.

shown that the capability to detect RV RNA exists in many countries and expertise for genotyping is available in the EUR. The lack of molecular data might be caused by the proportion of laboratory-investigated cases and cases confirmed by detection of the RV genome being too low.

In the elimination phase, it is required that the geographical and temporal distribution of the predominant virus sequence variants is continuously recorded and linked to additional relevant epidemiological information. The high complexity of molecular epidemiological data implies the need to create a methodology for the visualization of transmission patterns. The phylogenetic relationship between virus variants should be taken into account when transmission chains are analysed in the context of the EUR. Sequencing of additional genes or the whole viral genome could confirm a line of descent that is otherwise only presumed on the base of the sequence minimum routinely determined for genotyping [42–44]. For a genetic lineage with continuous detection in the EUR, the analysis of the global distribution using the nucleotide surveillance databases allows us to ascertain whether the main circulation area is inside or outside the region. Almost exclusive detection in the EUR, as recently observed for the presumed MV lineage 'D8-Frankfurt Main-Republic of Komi-Rostov on Don' would indicate that the transmission chain is maintained in the region without virus importation from outside.

Wide spread of certain MV variants across Europe by travellers, mobile communities or refugees has been reported recently [26,32,34,35,45]. Multiple importation of the same sequence variant by persons from the same geographic area indicates an ongoing outbreak in the country of origin or a country of transit. In the case of refugees, the collection of information on patient's history and travel route is often hampered by fear of disclosing details that might lead to deportation, social circumstances or the language barrier. Rapid reporting of the available molecular epidemiological information on virus importation will help to track the virus transmission route and recognize ongoing outbreaks at an early stage.

Molecular surveillance in the EUR is integrated in the global surveillance coordinated by the GMRLN that facilitates intercontinental tracking of virus transmission pathways [6,36,45]. Furthermore, genetic data describing virus variants that have been imported from endemic areas outside the EUR can help to fill surveillance gaps in these areas. The globally recorded molecular data demonstrate that the number of circulating MV genotypes has decreased to six (B3, D4, D8, D9, G3 and H1) detected in 2015 [7]. A continued disappearance of MV genotypes documented by a well-performing global surveillance would indicate further progress of the vaccination programme and supports the notion that eradication of measles is feasible.

Transparency declaration

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