Status of Global Virologic Surveillance for Rubella Viruses

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The suspected measles case definition captures rubella cases. Therefore, measles surveillance will be improved in the course of the control and eventual elimination of rubella transmission. One aspect of rubella control, virologic surveillance, is reviewed here. A systematic nomenclature for rubella viruses (RVs) based on 13 genotypes has been established and is updated when warranted by increases in information about RVs. From 2005 through 2010, the genotypes of RVs most frequently reported were 1E, 1G, and 2B, and genotypes 1a, 1B, 1C, 1h, 1j, and 2C were less frequently reported. Virologic surveillance can support rubella control and elimination. Synopses of rubella virologic surveillance in various countries, regions, and globally are given, including characterization of viruses from imported cases in a country that has eliminated rubella and studies of endemic viruses circulating in countries without rubella control objectives. Current challenges are discussed.

Measles and rubella are similar rash illnesses that may be difficult to differentiate clinically [1]. Measles control can be impeded when the incidence of rubella results in a substantial number of suspected measles cases that are ultimately classified as rubella cases [2]. Since major rubella epidemics in unvaccinated populations can be separated by 6–9 years, the annual impact of rubella cases on measles surveillance is difficult to predict (Figure 1) [2]. For example, in the country of Georgia, rubella annual incidence rates showed peaks in 1983, 1985, 1988, 1997, and 2004. The 2004 outbreak coincided with a measles outbreak; during this outbreak, 53% of suspected measles case patients tested positive for immunoglobulin M (IgM) to measles virus and 12% tested positive for IgM to rubella virus (RV). Some surveillance programs in support of measles elimination have integrated measles and rubella surveillance, with the final classification of suspected cases determined largely by laboratory results [3, 4]; such integration is cost-effective [5].

After measles elimination, a rubella control or elimination program, including rubella surveillance, can be

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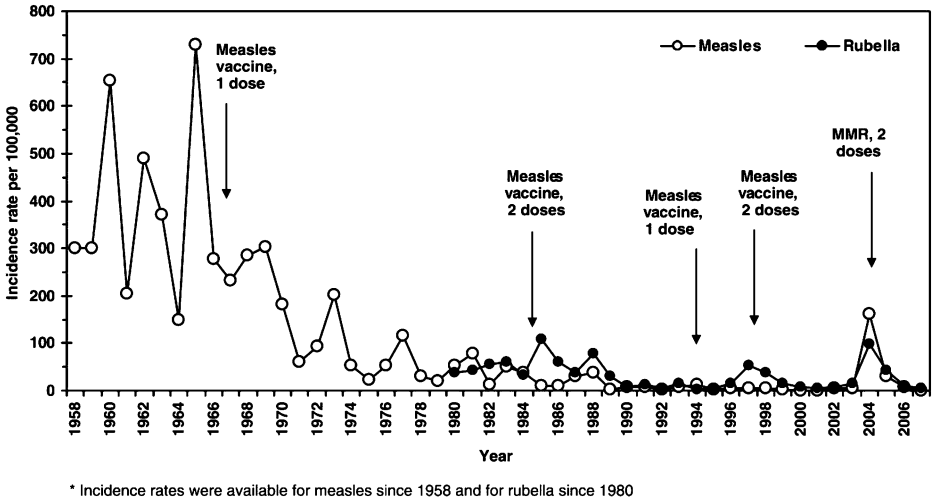

Figure 1. Measles and rubella incidence rates, Georgia, 1958–2007. Reprinted with permission from [2]. MMR, measles-mumps-rubella vaccine.

a catalyst for maintaining measles elimination [6]. This catalytic effect can happen when combined measles and rubella vaccines are used and when high-quality laboratory-based surveillance for rubella, which tests rubella-negative cases for measles, is used [7, 8]. There are rubella and congenital rubella syndrome (CRS) elimination goals and accelerated rubella control programs in many countries (see below).

Laboratory diagnosis of rubella is needed to confirm those rubella cases that are investigated in the course of measles surveillance due to the recommended use of a measles case definition that also captures rubella cases. The diagnostic assays and the clinical interpretations of laboratory results are sufficiently different for measles and rubella that laboratories must have specific experience in both diseases [9]. Two important techniques used in laboratory support for rubella diagnosis are the detection of rubella IgM and the detection of RV RNA by conventional and real-time reverse-transcription polymerase chain reaction assays [10]. Molecular epidemiology, defined here as virologic surveillance that allows the differentiation of circulating RVs, can be used to monitor transmission pathways and to identify interruption of endemic virus transmission [9]. The current status of virologic surveillance for circulating RVs worldwide is summarized here.

NOMENCLATURE

Rubella virus, the sole member of the *Rubivirus* genus in the Togaviridae family, is a positive-polarity RNA virus with a genome of 9,762 nucleotides. Five protein products are encoded by the genome: 2 non-structural proteins (P90 and P150) and 3 virion proteins (the capsid and 2 envelope glycoproteins, E2 and E1). A systematic nomenclature for RVs is necessary for effective virologic surveillance. The World Health Organization (WHO) Measles and Rubella Laboratory Network has recommended the collection of RV genotype data to support control and elimination programs globally [9]. Although rubella is a serologically monotypic virus, sequence analysis of the E1 glycoprotein revealed that distinct genetic variants of RVs exist [11]. In 2005, a systematic nomenclature was adopted by the WHO and has been described elsewhere [12–14]. Briefly, genetic characterization has identified 2 clades that differ by 8%–10% at the nucleotide level. Clade 1 is divided into 10 genotypes (1a, 1B, 1C, 1D, 1E, 1F, 1G, 1h, 1i, and 1j), of which 6 are recognized and 4 are provisional (designated by lowercase letters). Clade 2 contains 3 genotypes (2A, 2B, and 2C). All recognized genotypes must be represented by at least 2 well-characterized reference viruses for which the 3,192-nucleotide structural protein open reading frame (SP-ORF) has been sequenced. A 739-nucleotide region (nucleotides 8,731–9,469) within the SP-ORF was designated as the minimum sequence window necessary for assigning genotypes by comparison with the reference virus sequences. Figure 2 shows phylogenetic trees produced using 32 reference virus sequences. Accepted or proposed reference virus sequences are now available for all 13 genotypes. Genotype 1a virus sequences, currently represented by vaccine and laboratory viruses from the 1960s, do not cluster as a single group and should probably be further subdivided. Bootstrap values are robust for all genotypes except for 1D, using the SP-ORF sequences. The bootstrap values are less robust for some genotypes by means of the 739-nucleotide sequence window. The criterion for a valid analysis using 739-nucleotide sequences is the proper clustering of reference viruses, not bootstrap values [12].

OVERALL STATUS OF VIROLOGIC SURVEILLANCE FOR RVs

Global Distribution of RVs

Four of the 13 rubella virus genotypes were not reported from January 2005 through May 2010. Genotype 2A viruses have not been isolated since 1980, except for several isolates of the Chinese 2A vaccine strain, BRDII, from vaccine-associated

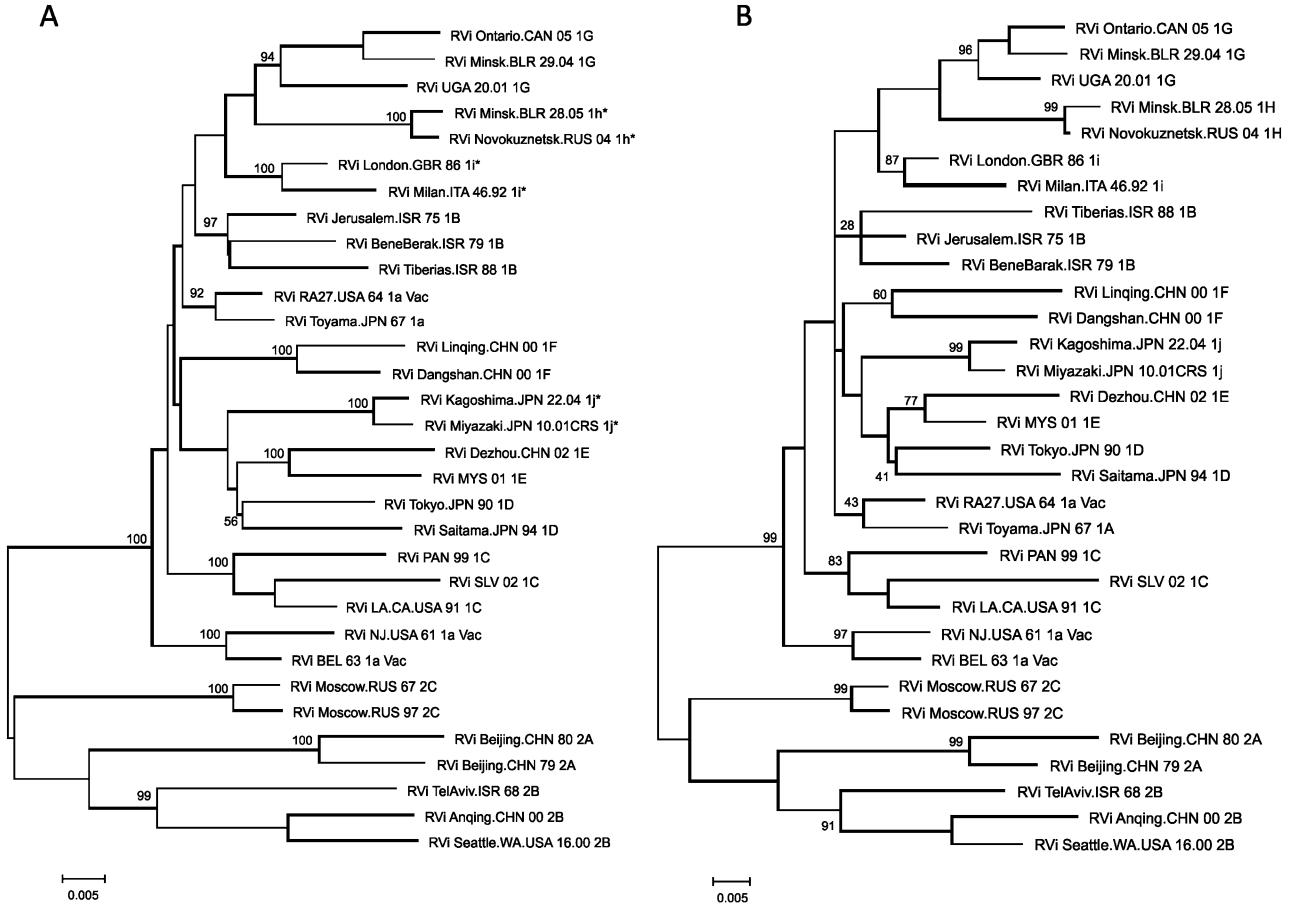

Figure 2. Phylogenetic analysis of 32 rubella reference virus sequences. Phylogenetic analyses were conducted using MEGA4 Software (version 4.0) [15]. The evolutionary history was inferred using the neighbor-joining method [16]. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches for each genotype. The branch lengths are in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura 3-parameter method [17] and are in units of the number of base substitutions per site. *A,* Phylogenetic tree based on the rubella structural protein coding region (3,192 nucleotides in length). Proposed reference virus sequences are marked with asterisks (E. Abernathy, unpublished data). *B,* Phylogenetic tree based on the 739-nucleotide region recommended by the World Health Organization for genotyping of rubella viruses.

cases. Viruses of genotypes 1i and 1F were last found in Italy (1994) and China (2002), respectively [18, 19]. Genotype 1D has not been detected since 1996. In 2007, 1j was determined to be a provisional genotype [13]. Some viruses that are now classified as 1j were considered to belong to genotype 1D before genotype 1j viruses were defined (see Figure 2). Therefore, care should be taken when working with virus sequences identified as genotype 1D before 2007. Reclassification of additional viruses by subdivision of some current genotypes (eg, genotypes 1E, 1G, and 2B) will likely be necessary to enhance the utility of RV molecular epidemiology.

The geographic distribution of the 9 active genotypes during the observation period is shown in Table 1 (GenBank; WHO database; D. Featherstone, personal communication). In some cases there are insufficient data to classify these viruses as either endemic or imported. Of these 9 genotypes, 6 (1a, 1B, 1C, 1h, 1j, and 2C) were reported sporadically or in geographically restricted regions. For example, viruses of genotype 1h were detected only in Russia and neighboring countries during the observation period [20], and genotype 2C was reported only from Perm, Russia (GenBank). The decline and likely elimination of viruses of genotype 1C from the Americas has been monitored by virologic surveillance, and no virus of this genotype has been reported for almost 5 years (Table 1) [14].

Viruses of the remaining three genotypes, 1E, 1G, and 2B, had a wide geographic distribution and were frequently found. Genotype 1E viruses have been identified in 1 Middle Eastern country, 7 European countries, 3 Southeast Asian countries, 3 African countries, and 4 Western Pacific countries, and are the dominant genotype in China [19]. Viruses of genotype 1G were found in 5 European countries and 7 African countries. Viruses of genotype 2B were reported from 2 Middle Eastern countries, 5 European countries, 4 Southeast Asian countries, 4 South and

Table 1. Global Distribution of Reported Rubella Virus Genotypes, 2005–2010

Genotype, country	Year or years
1a	
Cambodia	2009
Japan	2008
Kazakhstan	2006
1B	
South Africa	2007, 2008
1C	
Chile	2005
Peru	2005
1E	
Belarus	2005, 2006
China	2005, 2006, 2007, 2008,[a] 2009, 2010[a]
China HK SAR	2008, 2009, 2010
France	2005[a]
Kazakhstan	2006
Laos	2009
Malaysia	2005[b]
Mongolia	2010
Poland	2007, 2008[b]
Russia	2005, 2006, 2007, 2008, 2010
South Africa	2008
Sri Lanka	2008
Sudan	2005
Thailand	2005, 2009
Tunisia	2008
Ukraine	2007
United Kingdom	2008
United States	2008[c]
Vietnam	2007[b]
Yemen	2008
1G	
Algeria	2007
Belarus	2005
Ghana	2005, 2008
Côte d'Ivoire	2005,[b] 2008
Kenya	2005, 2010[b]
Libya	2009
Netherlands	2005[a]
Russia	2005,[b] 2006, 2008
Sudan	2005
Uganda	2007[b]
Ukraine	2009
United Kingdom	2007
1h	
Belarus	2005, 2006
Kazakhstan	2008[a]
Kyrgystan	2009
Russia	2005, 2006, 2007, 2008, 2009, 2010
1j	
Brazil	2005[c]
Philippines	2010[a]
Spain	2005[c]
United Kingdom	2006[c]
United States	2010[c]
2B	
Argentina	2008
Bangladesh	2009
Bosnia and Herzegovina	2009, 2010
Brazil	2006, 2007, 2008, 2009
Chile	2007
China	2008
China HK SAR	2008, 2009
Dubai	2009[b]
Egypt	2007[b]
France	2009
India	2005, 2007, 2008,[b] 2010[b]
Italy	2008[b]
Japan	2007[c]
Kazakhstan	2008[bc]
Mexico	2008[b]
Nepal	2008, 2009, 2010
Russia	2009[c]
South Africa	2007, 2008
Spain	2009
Sri Lanka	2008
Sudan	2006
Ukraine	2010
United Kingdom	2006, 2007, 2008, 2010
United States	2007,[c] 2009,[c] 2010[c]
Vietnam	2006,[b] 2009
Yemen	2008
2C	
Russia	2005

NOTE. Genotypes 1D, 1F, 1i, and 2A were inactive during this period. Country (or countries) and year (or years) of report are indicated for each genotype. HK SAR, Hong Kong Special Administrative Region.

[a] Found endemically and as an export.

[b] Exported virus; importation countries are shown in Table 2.

[c] Probable import, but links are unknown.

Central American countries, 3 African countries, and 2 Western Pacific countries.

Limited Number of RVs From Virologic Surveillance and Systematic Reporting of Sequences

There are a total of 647 virus entries in the WHO rubella virus genotype database for viruses found from 1966 through June 2010, and only 534 of these viruses have been sequenced through the 739-nucleotide window required for genotyping. GenBank contains rubella sequences, but these sequences are not evaluated for accuracy and updated when new information becomes available (eg, new genotype assignments) and often lack epidemiologic linkages. In addition, no dedicated database of rubella

sequences is currently available; the WHO genotype database currently does not provide sequence information apart from linkages to GenBank accession numbers, when the submitter provides them. Furthermore, there are still many countries that lack sufficient baseline data on circulating viruses, and there are only a few countries where RVs have been sampled over time [18–21].

CURRENT STATUS OF VIROLOGIC SURVEILLANCE FOR RVs IN THE WHO REGIONS

The WHO Region of the Americas

The member states of the Pan American Health Organization are working to complete the goal of elimination of rubella and CRS [22]. Using routine immunization, supplemental immunization activities (SIAs), and an integrated surveillance system, endemic rubella and CRS cases in the Americas are now at an all-time low [23, 24]. One notable recent achievement was work to eliminate rubella and CRS from Brazil by the administration of ~70 million doses of rubella-containing vaccine, which targeted women and men aged 20–39 years in all states and adolescents aged 12–19 years in 5 of 27 states [25].

Virologic surveillance has been important to elimination efforts in the Americas. Country-specific data from virologic surveillance in Canada and the United States supported the documentation of elimination of endemic rubella in these 2 countries [14]. The available information on RVs of genotypes 1C, 1E, 1G, 1B, 1j, and 2B found in the Americas from 1997 through 2007 has been recently summarized [14]. Viruses of genotype 1C (first identified in about 1983) have previously been found circulating only in the Americas, have not been found anywhere in the world since 2005, and are thus likely eliminated [24]. Prior to 2006, all genotype 2B viruses identified in the Americas were associated with importation events. In 2006, a virus or viruses of genotype 2B caused a large epidemic, predominantly in Brazil and Chile, which lasted for >1 year and thus met the criterion for endemic circulation [26, 27]. However, good virologic surveillance efforts after 2006 provided evidence for the decline and likely elimination of endemic viruses of genotype 2B in the Americas [24].

As for the other rubella virus genotypes previously found in the Americas, viruses of genotypes 1E and 1G are distributed widely in the world and viruses of genotype 1B have been found occasionally throughout the world from 1972 through 2008. Viruses of genotypes 1B and 1G were found in the Americas (mostly in Brazil) over a 7-year time span, indicating that these viruses were likely endemic. Viruses of genotype 1E may also have been endemic, but they were found only from 1997 through 1999, which would also be compatible with multiple importations. Furthermore, the period 1997–1999 was before major rubella control activities in most countries in the Americas, so if genotype 1E was endemic, its disappearance after 2 years would be surprising. Viruses of genotype 1j have only been found in the Americas as either known or likely imports [42, M. M. Siqueira and E. Abernathy, unpublished data].

The WHO European Region

The WHO European Region (EUR) has established a rubella elimination and CRS prevention goal by 2015 [5]. Among the different countries in this region, there are considerable differences in strategies and approaches to rubella control and rubella and CRS surveillance. In addition to different vaccination schedules with different kinds of rubella-containing vaccines and implementation of SIAs [28], many different reporting systems are currently in place. In some countries, reporting is mandatory for both rubella and rubella in pregnancy/congenital rubella infection (CRI)/CRS cases; in other countries, it is mandatory only for rubella cases or only for rubella in pregnancy/CRI/CRS cases. Some countries rely on voluntary laboratory-based reporting systems or operate supplementary sentinel systems [29]. In addition, there are discrepancies related to whether clinical cases or only laboratory-confirmed cases are reported, further complicating comparisons of reported cases between countries [29–31].

The quality and sensitivity of surveillance differs between countries, but compared with the total number of reported cases, the number of laboratory-confirmed cases and/or cases with known epidemiological links or importation status still seems low (WHO database; D. Featherstone, personal communication) [30]. The majority of rubella cases seem to occur among unvaccinated individuals, and there are still many cases reported among individuals ≥20 years old [29–31].

From 2007 through 2009, WHO received aggregated case numbers from 49 of the 53 EUR states [32], but data on virus genotypes were available from only 7 countries (WHO database; D. Featherstone, personal communication). Both WHO and EUVAC.NET data from this period indicate that the bulk of cases were reported from only few countries and that several countries reported few or no cases from 2007 through 2009 [29–32]. Some countries in the region may have achieved rubella elimination, whereas others are still far from realizing this goal.

Little or no baseline information on endemic rubella virus genotypes is available from most EUR countries. Sufficient information on whether a case is endemic or imported may not be collected, and thus it is sometimes difficult to classify viruses as endemic or imported. From 2007 through 2009, reports of 88 RV genotypes (31 of genotype 1h, 24 of genotype 1E, 21 of genotype 2B, and 12 of genotype 1G) from 7 different countries in the EUR (Russia, United Kingdom, Kazakhstan, Bosnia and Herzegovina, Poland, Ukraine, and Kyrgyzstan) were submitted to the WHO genotype database, most of them from Russia ($n = 51$) and from the United Kingdom ($n = 20$) (WHO database; D. Featherstone, personal communication). RV sequence information for ~300 sequences from 18 EUR countries is

available on GenBank. For several years, viruses of genotypes 1E, 1h, and 1G were among the most widespread in the EUR (WHO database; D. Featherstone, personal communication) [20, 21, 33, 34], whereas in the United Kingdom, many cases of genotype 2B were registered (WHO database; D. Featherstone, personal communication) [35]. The latter genotype has lately been identified more often in other EUR countries (eg, Bosnia and Herzegovina, Kazakhstan, Russia, and France) both from sporadic cases and from outbreaks (WHO database; D. Featherstone, personal communication) [21, 36].

The WHO Eastern Mediterranean, Western Pacific, African, and South-East Asian Regions

Overall, countries in the Eastern Mediterranean Region (EMR), Western Pacific Region (WPR), African Region (AFR), and South-East Asian Region (SEAR) of WHO have significantly improved virologic RV surveillance in recent years. There are considerable differences in the extent of rubella control and elimination programs among the countries within these regions. The WPR has an accelerated rubella control and CRS prevention objective [37]. The genotype of at least 1 RV has been determined from many countries in the AFR, EMR, SEAR, and WPR (Table 1). Most of these countries are working to establish genetic baseline data prior to embarking on significant rubella control and elimination efforts.

The strong virologic surveillance required in countries where rubella is controlled has often provided the genotypes of viruses from countries without strong rubella virologic surveillance. Such surveillance of imported cases has enabled the identification of the genotype of viruses in the country of exposure (Table 2) [WHO database; D. Featherstone, personal communication, 38–40]. This information has proved valuable in the exporting regions and at the global level. Thorough investigation of imported cases is important, since infections can occur in airports, in airplanes, or in transit centers [40].

Table 2. Reported Export and Import Pairs of Rubella Virus, 2005–2010

Exporting country, importing country	Genotype	Year
China		
United States	1E	2008
United Kingdom	1E	2010
Dubai		
Canada	2B	2009
Egypt		
Canada	2B	2007
France		
United Kingdom	1E	2005
India		
United States	2B	2008, 2010
Italy		
United Kingdom	2B	2008
Côte d'Ivoire		
United States	1G	2005
Kazakhstan		
Russia	1h	2008
United Kingdom	2B	2008
Kenya		
United States	1G	2010
Malaysia		
United States	1E	2005
Mexico		
United States	2B	2008
Netherlands		
Canada[a]	1G	2005
Philippines		
China HK SAR	1j	2010
Poland		
United Kingdom	1E	2008
Russia		
United Kingdom	1G	2005
Uganda		
United States	1G	2007
Vietnam		
China	2B	2006
China (Province of Taiwan)	1E	2007

NOTE. The criteria for establishment of epidemiological links may not be consistent between pairs. HK SAR, Hong Kong Special Administrative Region.

[a] This importation led to a large outbreak in Canada (see text for further discussion).

SPECIFIC EXAMPLES FROM CURRENT VIROLOGIC SURVEILLANCE

Virologic Surveillance in a Country That Has Eliminated Endemic RVs and CRS

In the United States, the Centers for Disease Control and Prevention reported in 2005 the results of an expert review panel that considered the status of rubella and CRS elimination in the United States [41]. Based on a review of the history of the rubella vaccination program in the United States, epidemiology, molecular epidemiology, seroprevalence, vaccine coverage, and adequacy of surveillance, the panel concluded that RV was no longer endemic in the United States [42]. A recent summary of all available data provided evidence that elimination of endemic rubella has been maintained. The data in support of maintenance of elimination included a virologic surveillance profile of rubella and CRS that showed a pattern of viral genotypes consistent with viruses originating outside the United States [43].

Since rubella and CRS have been eliminated from the United States, identified cases receive intense investigation, including, when possible, determinations of the genotypes of the viruses. From 2007 through 2010, the genotypes of viruses from 14 cases found in the United States were determined to be 2B (9 cases), 1E (2 cases), 1G (2 cases), and 1j (1 case). Viral RNA was

detected in a specimen from 1 additional imported case, but insufficient RNA was available to determine a genotype. Genotypes of 7 of these viruses were consistent with the epidemiologic data about the country of exposure to rubella (India, China, Uganda, Kenya, and likely Mexico), and 7 cases did not have epidemiologic data indicating a country of exposure. Although good sequence information from these 7 viruses was available (5 genotype 2B viruses, 1 genotype 1E virus, and 1 genotype 1j virus), there is insufficient baseline data on RVs of these genotypes in the world to allow assignment of country of exposure on the basis of sequence information alone. As expected, documented cases of CRS occurring in the United States as the result of exposures in other countries have been found after 2005 [38].

Use of Virologic Surveillance in Regions Seeking to Certify Rubella Elimination

In countries or regions where rubella and CRS control has been achieved, molecular epidemiological data can help to support the process of certification of rubella and CRS elimination, especially if comprehensive baseline data are available for comparison. As described above, the Americas have adopted a rubella elimination goal, and virologic surveillance data will certainly contribute to certification of elimination. Tracking the elimination of viruses of genotypes 1C and 2B as well as virologic investigations of imported cases (Table 2) will likely be key contributions to certification [44]. The EUR of WHO is working toward a similar use of virologic surveillance data.

Use of Virologic Surveillance in a Country With Many Importations

Frequent importations of RVs pose challenges for some countries that are trying to eliminate rubella. Molecular epidemiological data obtained in such settings can help to distinguish between endemic and imported viruses. In the United Kingdom, for instance, most rubella cases during the past years were caused by genotype 2B viruses. Importations of viruses of genotypes 1E (eg, from Romania, France, Poland, and China), 1G (eg, from Africa and Russia), 1j (from the Philippines), and 2B (eg, from Italy and maybe Kazakhstan) were documented during the past 8 years, mostly to London [40].

Collection of Baseline Virologic Data in Countries With Endemic Rubella

Some countries are collecting rubella baseline data. In China, for instance, rubella vaccination was included in the national immunization program only in 2007, and large-scale investigation of RVs collected from 1979 through 2007 provided valuable information from the prevaccination era [4]. Five different genotypes (1a, 1E, 1F, 2A, and 2B), some of which cocirculated, were documented in China (eg, 1a and 2A from 1979 through 1984, 1F and 2B from 1999 through 2000, and 1F and 1E from 2001 through 2007). In Anhui province, genotype 1F and 2B viruses were likely replaced by 1E viruses in connection with an epidemic in 2001. From 2001 through 2007, genotype 1E viruses were clearly predominant in the 17 of 31 provinces surveyed in China, indicating that a similar genotype shift likely took place in other provinces [19]. Additional examples of countries with recent baseline data collections include Italy [18], Russia [34, 45], Japan [46], Belarus [20], and France [21].

Use of Virologic Surveillance to Document the Spread of Rubella Outbreaks

Molecular epidemiological data can be used to document importation followed by an outbreak or sustained circulation in the new location. In 2004–2005, a rubella outbreak was reported in the Netherlands, which spread to Canada, where it caused sustained transmission for a prolonged period [33]. In total, 387 cases were reported in the Netherlands and 309 cases were reported in Canada; 97% of cases in both countries were among unvaccinated people belonging to orthodox Protestant communities. RV isolates obtained in the Netherlands and in Canada both belonged to genotype 1G and were closely related. These data were consistent with the spread of rubella from the Netherlands to Canada despite the lack of a known contact between cases in the Netherlands and the first cases in Canada [33].

SUMMARY AND CHALLENGES

Virologic surveillance has contributed significantly to major control and elimination programs for polio and measles [47, 48]. The suspected measles case definition also captures rubella cases. Therefore, measles surveillance will be improved in the course of the control and eventual elimination of rubella transmission. An important part of rubella surveillance is virologic surveillance for RVs.

Data from virologic surveillance for RVs contributed to the program to document the elimination of rubella and CRS from the United States and has already contributed to major rubella control programs in the Americas and Europe [42]. However, the lack of comprehensive rubella surveillance in other parts of the world, the paucity of RVs that have been sequenced, and the lack of an established sequence database for RVs limits the utility of data from virologic surveillance for RVs. It has not been possible to test the utility of virologic surveillance data in a number of situations, including tracking of the source of imports with sequence data alone (eg, some viruses imported into the United States and United Kingdom) and identification of the source of major outbreaks with sequence data alone (eg, the 2006 epidemic in Brazil). The current lack of understanding of the dynamics of RV circulation in unvaccinated populations is due to limited virologic surveillance over time [19]. Furthermore, a better understanding of the molecular evolution of RVs over time should allow rigorous assignment of RVs to genetic groups and determinations of other key epidemiologic

information (eg, duration of endemicity of specific RVs in a given location), as has been done for other viruses [49].

There have been significant efforts in virologic surveillance in countries that have eliminated rubella, in countries that seek to eliminate measles and rubella, and in some countries with no rubella control goals that are seeking to eliminate measles. However, considerable efforts to strengthen virological surveillance of rubella are needed to fully exploit data of this type in support of elimination efforts.

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