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Rubella and congenital rubella syndrome (CRS) continue to be important health problems in many countries. In June 2004, the World Health Organization Steering Committee on Research Related to Measles and Rubella Vaccines and Vaccination met to evaluate data from research and operational activities and to identify critical scientific issues and gaps in knowledge that need to be addressed to improve the global control of rubella and CRS. Information about surveillance for rubella, natural and vaccine-induced immunity to rubella, laboratory diagnosis, the molecular epidemiological profile of rubella virus, and mathematical modeling to assess the burden of CRS and the impact of rubella vaccination was reviewed. This report summarizes the presentations and recommendations for future research.

The World Health Organization (WHO) conducted a worldwide survey on rubella, congenital rubella syndrome (CRS), and uptake of rubella vaccine during 1995–1996 [1, 2]. This survey reported an incidence of CRS of 0.6–2.2 cases/1000 live births during epidemics in developing countries, a rate similar to those of industrialized countries before vaccination. The problem of CRS has been largely overlooked in many developing countries, where it is a significant cause of deafness, blindness, and mental retardation. Difficulties arise in surveillance of CRS, because all 3 organ systems are not always affected and the clinical manifestations during the first year of life may vary [3]. For example, the proportion of children with CRS who have hearing loss may be as high as 70%, but detecting hearing deficit in infants and young children depends on the diagnostic methods available. Accurate case identification is also problematic, because definitive laboratory tests for CRS have variable sensitivity that depends on the age of the child.

The challenges of accurate diagnosis of CRS and complete assessments of the consequences of maternal rubella during pregnancy have complicated public-health estimates of CRS and, therefore, estimates of the potential benefit of rubella vaccination programs to the health of children. Nevertheless, an economic analysis of rubella vaccination—incorporating data from 22 studies, including 10 reports from developing countries—indicated that the benefit-cost ratio for rubella vaccination was similar to that for hepatitis B and Haemophilus influenzae type b vaccination [4].

RUBELLA VACCINES AND VACCINE-INDUCED IMMUNITY

Although several attenuated rubella vaccines were developed in the 1960s, the RA27/3 vaccine is now used
in most countries, often in combination with measles or measles-mumps vaccines. Rubella vaccines are well tolerated, and immunization induces seroconversion in 95% of vaccinees [5]. Hemagglutination inhibition antibodies usually develop 10–28 days after vaccination, and antibodies are produced to the structural proteins E1, E2, and C. Responses to RA27/3 rubella vaccine are similar to responses to natural infection, but antibody levels are lower. Some vaccinees lose detectable antibodies to rubella virus some years after vaccination but respond to re-vaccination with an anamnestic secondary immune response. Challenge studies show that very low levels of antibodies to rubella virus appear to be associated with susceptibility to re-infection but have not identified any antibody level or antibody subclass that correlates with protection. Protective antibodies are probably directed to conformational epitopes of rubella virus proteins, although the critical targets have not yet been defined. Rubella virus–specific T cells are elicited, but the role of cell-mediated immunity in protection is not understood. Similar antibody responses to rubella virus are elicited when RA27/3 vaccines are administered by subcutaneous and intranasal routes.

In developed countries, vaccination of susceptible pregnant women against rubella has not resulted in CRS in their infants [6]. Several large-scale studies of the consequences of inadvertent rubella vaccination during pregnancy are being conducted in the Americas (Costa Rica, Brazil, El Salvador, Ecuador, and Paraguay). Preliminary results reported by R. Castalia Soares (personal communication) from a study in Brazil during 2001–2002 showed that 2327 pregnant women were susceptible to rubella when vaccinated. Of 1759 infants born to these women and tested for rubella virus–specific IgM, 3.6% had laboratory evidence of congenital rubella virus infection and none had CRS.

The development of an aerosol measles vaccine has generated interest in designing an aerosol rubella vaccine that could be administered as a combined measles-rubella vaccine. To consider this proposal, WHO organized the Consultation on Immunization against Rubella by the Aerosol Route in April 2004 in Geneva. Initial studies have demonstrated that this type of vaccine has good immunogenicity. Evaluation of a combined measles-rubella aerosol vaccine administered to healthy school-aged children in Mexico showed that the aerosol route was more immunogenic than the subcutaneous route for measles vaccine and was equally immunogenic for rubella vaccine, whereas for both antigens the aerosol vaccine was less reactogenic [7]. A second study in Mexico showed that reactogenicity was lower in recipients of the aerosol vaccine, and RA27/3 rubella vaccine was more immunogenic when administered by the aerosol route than by the subcutaneous route [8]. The potential benefit of combining an aerosol rubella vaccine with a measles vaccine include ease of administration and cost savings.

**REDUCING THE BURDEN OF CRS IN THE WHO REGION OF THE AMERICAS**

As part of a highly successful measles elimination strategy, the measles surveillance system in the WHO Region of the Americas began to document widespread circulation of rubella virus in many countries during the mid-1990s. In response, the Pan American Health Organization (PAHO) Technical Advisory Group on Vaccine Preventable Diseases recommended in 1997 that a regional initiative be implemented to strengthen prevention efforts against rubella and CRS [9]. Since the introduction of the rubella vaccine and vaccination campaigns for adults, the incidence of rubella has declined by 99.3%, from 135,000 reported cases during 1998 to 923 cases during 2003. Countries in the Region of the Americas recognize the significant benefit of the accelerated control of rubella and CRS [4, 10].

In September 2003, PAHO’s 44th Directing Council of Ministers of Health of the Countries of the Americas adopted a resolution to eliminate rubella and CRS by 2010 [11]. Elimination has been defined as the successful interruption of endemic transmission of rubella in all countries of the region without the occurrence of cases of CRS associated with endemic transmission [12]. For elimination purposes, full integration of measles and rubella surveillance is required, with an emphasis on active surveillance. To prevent infection of susceptible pregnant women, adult mass vaccination campaigns targeting both men and women in all countries of endemicity is proving to be a highly effective strategy for eliminating transmission of rubella. In each country, the age group to be vaccinated is determined by the year of introduction of rubella vaccination, the timing of follow-up vaccination campaigns to maintain elimination of measles (using measles-rubella or measles-mumps-rubella vaccine), the epidemiological profile of rubella, and the reproductive health practices of the country.

As of November 2004, ~99% of new birth cohorts in the Region of the Americas have access to the measles-mumps-rubella vaccine. Only Haiti has yet to include the vaccine in its vaccination schedule. Between 1998 and November 2004, 72% of countries, representing 66% of the population of the region, have implemented supplementary immunization activities targeting adults (figure 1). Vaccination coverage rates achieved in the Caribbean reached 80%, and most of the other countries had coverage rates of >95% [13].

During 2003, the regional surveillance system for rubella and measles reported 34,766 suspected cases. Of these, 105 cases of measles and 713 cases of rubella (77%) were confirmed by laboratory testing, with an additional 210 cases of rubella confirmed by epidemiological links.

In the Western Hemisphere, it was estimated by mathematical modeling that 20,000 infants with CRS were born during 1997, before widespread use of rubella vaccine in the region.
Figure 1. Countries in the World Health Organization Region of the Americas with elimination programs for rubella and congenital rubella syndrome, 1998–2004. Data on the use of the measles-mumps-rubella vaccination are from country reports.

[14]. The number of countries (or territories) in the Region of the Americas that reported suspect cases of CRS increased from 18 (41%) during 1998 to all countries during 2003. Countries in the Region of the Americas reported 44 confirmed cases of CRS during 1998, 63 during 1999, 90 during 2000, 41 during 2001, 24 during 2002, and 14 during 2003. Some countries have conducted retrospective studies in community obstetrics hospitals and in special schools for the deaf and blind to identify children with probable or confirmed CRS (table 1) [15–18]. Strengthening of health services through the implementation of higher-quality and more-sensitive laboratory methods—such as rubella virus-specific IgM testing, RT-PCR, and/or viral isolation—is needed. Rubella virus isolation and genotyping data are also very useful for tracking transmission pathways, investigating suspected postvaccination cases, documenting the elimination of endemic strains, and providing additional data confirming the importation of cases from other regions. Improved health services, particularly for women, is expected to eliminate rubella and CRS in the Region of the Americas [19, 20].

REDUCING THE BURDEN OF CRS IN THE WHO EUROPEAN REGION

The WHO European Region has a population of ~880 million. Rubella is a nationally reportable disease in all but 7 of 52 countries in the European Region. The number of cases of rubella reported annually to the WHO Regional Office for Europe has remained fairly stable over the past decade, with 304,320 cases reported during 2003 [21]. The cost of laboratory testing of clinical specimens with kits of suitable quality has been identified as an important impediment to strengthening rubella surveillance.

Ninety-seven cases of CRS were reported to the WHO Regional Office for Europe during 2000–2003. It is recognized that these data represent marked underreporting. Although the percentage of countries that reported cases increased from 71% during 2000 to 94% during 2003, cases were reported from only 11 countries, and 37 (36%) were reported from Romania, a country with only 2.6% of the European Region’s population [22]. In Kyrgyzstan, the annual incidence of CRS was estimated, on the basis of a seroprevalence study, to be 12 cases/100,000 live births [23]. A study performed in Perm, Russia, found an incidence of CRS of 3.5 cases/1000 live births [24]. In Italy, national surveillance for CRS was discontinued during 1992, but regional outbreaks of rubella with associated cases of CRS have occurred [25]. Although surveillance for CRS has been performed effectively through traditional disease-reporting mechanisms [22] and pediatric surveillance units [26], further work is necessary to identify optimal methods for detecting cases in countries with different capacities of their health systems.

During 1998, the European Region approved the target of <1 case of CRS/100,000 live births by 2010 [27]. To meet this challenge and to achieve elimination of measles, a measles and CRS strategy was developed during 2002 [28–30]. The approach has been to closely link measles and rubella prevention activities by strengthening routine 2-dose measles-containing vaccine programs and by using opportunities provided by supplementary immunization activities, including immunization safety practices, to gain access to difficult-to-reach and susceptible populations of children and adults and further strengthen these programs.

The use of rubella vaccine has markedly increased since 2002 in the European Region. Forty-seven countries (90%), with 86% of the population, now use it, mostly in the form of the measles-mumps-rubella vaccine (figure 2); however, those in the eastern part of the European Region have only recently introduced the vaccine, and some countries in Western Europe, where vaccination has been used for a longer time, have historically had inadequate coverage rates for preventing outbreaks of rubella and protecting women of childbearing age [21, 31, 32].

Supplementary immunization activities against rubella in the European Region are summarized in table 2. Measles-rubella vaccine is being used to target cohorts susceptible to measles and rubella, and rubella vaccine is given to women of childbearing age; the upper age limit is set to include at least 80% of women giving birth. Rubella-susceptible women who have immigrated into the European Region have been identified as an important target group [33, 34]. Programs to immunize these women will be necessary even after elimination of rubella has been achieved, because they may give birth to an infant with CRS after acquisition of infection outside of the region.
Table 1. Results of retrospective studies of the burden of congenital rubella syndrome in selected countries.

<table>
<thead>
<tr>
<th>Country (year of study)</th>
<th>Birth cohort</th>
<th>Methodological approach</th>
<th>Cases with final classification, no.</th>
</tr>
</thead>
</table>

NOTE. A clinical case was defined as the presence of 2 clinical manifestations (major criteria) or 1 principal and 1 associated manifestation (minor criteria) detected; a confirmed case was defined as the presence of rubella virus–specific IgM and signs consistent with a diagnosis of congenital rubella syndrome. Data are from [15].

In summary, in both the WHO European Region and the Region of the Americas, strategies for rubella elimination have evolved to include the combined measles-rubella vaccine and integrated approaches for surveillance for measles and rubella. The effectiveness of these regional approaches toward eliminating CRS and strengthening routine immunization services and health services needs to be documented. In both regions, high vaccine coverage rates among children and adolescents of both sexes is sought; however, there are regional differences with regard to vaccination of adults. The other 4 WHO regions have not yet established a rubella control goal. Further work needs to be done to identify the optimal methods for detecting cases of CRS, particularly in low- to middle-income countries, including an assessment of the sensitivity, specificity, and predictive value of the different clinical definitions of CRS.

LABORATORY METHODS FOR DIAGNOSIS OF RUBELLA AND CRS

Because the clinical diagnosis of rubella and CRS is difficult laboratory tests are required to confirm infection [6]. Rubella is usually diagnosed by detection of rubella virus–specific IgM in serum. Most commercial kits used for detection of rubella virus–specific IgM have high sensitivity and specificity, but specific IgM may not be detectable until >7 days after the onset of rash. As the true incidence of rubella becomes low, the positive predictive value of rubella virus–specific IgM tests for confirming recent infection declines. This circumstance is a particularly important issue in countries in which elimination of rubella has almost been achieved. As a consequence, testing for rubella virus–specific IgM in pregnant women is not recommended unless there is a history of rubella or contact with a person with a rubella-like illness, to avoid false-positive results. Other tests for confirming recent rubella virus infection include specific IgG avidity and reverse-transcription polymerase chain reaction (RT-PCR). However, neither of these methods has been subjected to comprehensive comparative studies to define sensitivity and specificity. A combination of rubella virus–specific IgM, IgG, and IgG avidity tests can be useful to confirm rubella virus infection during pregnancy.

Most studies of laboratory diagnosis of CRS were performed many years ago and focused on isolation of virus and rubella virus–specific IgM testing. IgM antibody to rubella virus can be detected in ~100% of cases in infants 0–3 months old, but, even with optimal testing methods, it is present in only ~57% of infants 6–12 months old [35]. Systematic studies are needed to assess modern methods, such as RT-PCR, for diagnosis of CRS. Oral fluid is an attractive specimen for diagnosis of rubella, because it is easily collected without invasive procedures and the pattern of antibody present in oral fluid is identical to that in blood. In the United Kingdom, oral fluid is the specimen of choice in testing for rubella virus–specific IgM. Oral fluid has also been used in testing for IgG specific for rubella virus, IgG avidity tests, and RT-PCR.

WHO has established the Measles/Rubella Laboratory Network, which includes 671 laboratories in 149 countries.
Table 2. Rubella vaccine supplementary immunization activities in the European Region, 2000–2003.

<table>
<thead>
<tr>
<th>Country or region, date/vaccine</th>
<th>Targeted group</th>
<th>Incidence of rubella after supplementary immunization activity, cases/per 100,000 population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albania</td>
<td>1–14 M, F</td>
<td>0.3</td>
</tr>
<tr>
<td>2000/MR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2001–2002/MR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kyrgyzstan</td>
<td>7–25 M, F</td>
<td>0.1</td>
</tr>
<tr>
<td>2001/MR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moldova</td>
<td>8–19a M, F</td>
<td>2004 data not available</td>
</tr>
<tr>
<td>2002/MR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003/rubella</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kosovo</td>
<td>1–15 M, F</td>
<td>2004 data not available</td>
</tr>
<tr>
<td>2003/MR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** MR, measles-rubella

* Persons 20–23 years old in organized settings such as universities and the military also received MR vaccine.

work training workshops focus on tests for measles, rubella, and yellow fever, where appropriate, by standardized testing methods that are quality controlled. The primary function of the network is to confirm suspected cases of measles and rubella by ELAs that test for rubella virus–specific IgM. The WHO Region of the Americas/PAHO reports laboratory data weekly, and the other WHO regions have begun or are planning to report such data monthly. Data from 25 African countries with case-based surveillance for suspected cases of measles show that rubella was the final diagnosis in >30% of cases in Angola, Benin, Ghana, Kenya, South Africa, Togo, and Zambia. Data from Shandong Province, China, indicate that 49% and 43% of suspected cases of measles were actually cases of rubella during 2002 and 2003, respectively, with a shift in the age distribution toward older ages.

**MOLECULAR EPIDEMIOLOGICAL DATA ON RUBELLA**

Molecular epidemiological data have been used to support control and elimination of vaccine-preventable diseases such as polio and measles. The discovery that vaccine-derived poliovirus recombinants can circulate and cause poliomyelitis contributed to understanding a poliomyelitis outbreak in Hispaniola that started during 2000 [36]. During 2001, an outbreak of measles in Venezuela and Colombia was caused by the imported measles virus genotype D9, rather than D6, which had caused previous outbreaks in the region [37, 38].

Phylogenetic studies of rubella viruses have identified 2 virus clades (formerly called genotypes), which differ in their nucleotide sequences by ~8%–10%. Each clade consists of a number of genotypes [39, 40]. A systematic nomenclature proposed at a WHO meeting in September 2004 divided the viruses into 2 clades (1 and 2), 7 genotypes (designated by clade and a letter designation, e.g., 1C), and 3 provisional genotypes [41]; this nomenclature is used here. Clade 2 viruses have not been found circulating in the Region of the Americas, and, thus, clade 2 viruses found in this region are considered to be importations [42]. Some rubella virus genotypes are geographically restricted (Figure 3). The number of viruses collected from populations at risk, such as those with low vaccination coverage rates, and from some large geographic regions is small [43, 44].

The WHO Measles/Rubella Laboratory Network often performs a significant amount of rubella testing even in areas without rubella control goals, because the differential diagnosis of measles and rubella is important in the control of measles [45]. Collection and transportation protocols for obtaining measles virus genetic material can usually be used for rubella virus [45]. Alternative collection protocols, such as the use of dried blood spots, may be suitable for obtaining rubella virus RNA [46]. Rubella virus RNA may be present in specimens lacking infectious virus, and, thus, use of direct RT-PCR is sometimes preferable for collection of molecular epidemiological data [47].

The development of Vero/SLAM cells for isolation of measles virus should facilitate the development of integrated measles and rubella virus isolation protocols, because both viruses replicate in these cells. Measles virus–negative cultures could be screened by indirect immunofluorescence for rubella virus, allowing the routine collection of circulating rubella viruses in network laboratories [48].

In summary, baseline genetic data on rubella viruses are needed from many countries and specific populations. Integrated protocols for measles and rubella virus are feasible and would allow the routine collection and transportation of rubella virus isolates and/or rubella virus genetic material. More stud-
Figure 3. The worldwide distribution of rubella viruses identified between 1985 and 2004 by genotype. Countries for which no genotype information is available are not shaded. Some genotypes are geographically restricted (e.g., the 1C virus found in Japan was considered to be an importation from the Region of the Americas). Because a lengthy period is covered, viruses presently circulating may be different. When the provisional status of some genotypes (1a, 1g, and 2c) is resolved, some viruses may be classified in other genotypes. An asterisk indicates that information for the virus was not obtained from the indicated country but instead was obtained from a country to which the virus was imported. The boundaries shown in this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city, or area of its authorities or concerning the delimitation of its frontier or boundaries. Reprinted with permission from [41].

ies of the diversity and dynamics of rubella virus, including sequencing of large portions of selected viruses, should support rubella control programs globally.

MODELING THE BURDEN OF CRS

Obtaining reliable estimates of the incidence of CRS from CRS surveillance studies is challenging, as discussed above. An alternative and practical method of estimating the burden of CRS in an unvaccinated population is based on estimates of the incidence of rubella virus infection among women of childbearing age from a cross-sectional age-stratified serological survey of antibody to rubella virus [49, 50]. Combining these results with local age-specific fertility data and the well-documented risks of CRS from maternal infection during pregnancy [51, 52] yields estimates of the incidence of CRS in the population [53]. Such estimates are reasonably robust to the point in the epidemic cycle at which the serological survey was conducted [54]. However, it is important to ensure that the rubella assay used is highly sensitive in adults—use of the conservative cutoffs recommended when screening for rubella immunity will produce many false-negative results, overestimate susceptibility to rubella, and give misleading estimates of the burden of CRS. Mixture modeling methods that exploit quantitative antibody results help to overcome this problem [55].

Introducing a vaccination program will affect the transmission of rubella within a population and alter the incidence of CRS. However, the impact is difficult to predict because the risk of infection for unvaccinated persons will be reduced when there are fewer persons with rubella who can infect them. Predicting these indirect “herd immunity” effects requires the use of dynamic models that explicitly describe transmission from infected to susceptible persons in the population. It has long been established, by such models, that introducing rubella vaccination for infants can eliminate transmission of rubella if high coverage rates are maintained but that low coverage rates can lead to an increase in susceptibility among women of childbearing age and an increase in the incidence of CRS in the medium to long term [56–58]. The impact within a particular country will depend on the prevaccination epidemiological profile of rubella: the higher the prevaccination transmission rate of rubella, the lower the burden of CRS and the higher the vaccination coverage rate needed to achieve a reduction in the incidence of CRS [59, 60].

WHO recommends that countries evaluating the introduc-
tions of rubella vaccination should consider 2 alternative approaches: selective vaccination to minimize susceptibility of women of childbearing age and elimination of rubella. The most appropriate strategy in any particular setting will depend on the prevaccination epidemiological profile of rubella (including the burden of CRS), the resources and capacity available, and the ability to sustain a program. The reassuring studies of the safety of vaccination during pregnancy (reviewed in [6]) suggest that widespread campaigns among women of childbearing age could be used as a stand-alone strategy in some settings. Such campaigns would have a marked and rapid impact on the incidence of CRS but would not carry the risk of catastrophic failure if they could not be sustained. This strategy and others should be considered in cost-effectiveness analyses that use dynamic transmission models to predict effectiveness in a range of scenarios. Cost-effectiveness studies (reviewed in [4]) that do not include indirect effects, which have a crucial impact on the effectiveness of rubella vaccination, are of little value [61].

SUMMARY OF RUBELLA RESEARCH AGENDA

Further research should contribute considerably to the eventual goal of rubella elimination and control of CRS. The WHO recommendations for rubella research are as follows:

- Improve surveillance for rubella and congenital rubella syndrome and summarize lessons learned
- Analyze cost-effectiveness of rubella vaccination strategies on the basis of the local epidemiological profile of rubella
- Evaluate laboratory diagnostic methods when rubella vaccination programs are introduced and in countries in which rubella has almost been eliminated
- Expand knowledge about the molecular epidemiological profile of rubella virus by testing viruses from unstudied geographic areas and populations with low vaccination coverage rates
- Evaluate rubella immunity after natural infection and vaccination to identify immune correlates of protection
- Evaluate aerosol rubella vaccine for combined use with aerosol measles vaccine
- Evaluate consequences of inadvertent rubella vaccination during pregnancy and in HIV-infected women
- Devise demographically realistic dynamic models with regional rubella surveillance data to guide vaccination strategies, project the incidence of congenital rubella syndrome, and assess achievement of policy goals for control of congenital rubella syndrome.

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