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Conference report

Reducing global disease burden of measles and rubella: Report of the WHO Steering Committee on research related to measles and rubella vaccines and vaccination, 2005

Abstract

The WHO Steering Committee reviewed and evaluated the progress towards global control of measles and rubella and provided guidelines for future research activities concerning both diseases during its meeting in New Delhi, in April 2005. Global measles vaccination coverage increased from 71% in 1999 to 76% in 2004 and indigenous transmission was interrupted or kept at very low levels in many countries. However, Africa and Southeast Asia continue to experience endemic transmission and high mortality rates, despite a global mortality reduction of 39% between 1999 and 2003. On the basis of reports from countries with continued indigenous measles virus transmission, future control strategies as well as advantages and potential drawbacks of global measles eradication were discussed. Similarly the burden of rubella and congenital rubella syndrome (CRS) as well as the cost-effectiveness of rubella vaccination was assessed using different methods in several countries without vaccination programs. As measles and rubella viruses continue to circulate surveillance and control strategies need further optimization. RT-PCR was considered as an alternative method for laboratory diagnosis of CRS. The value of dried blood spots and oral fluid as alternative samples for measles and rubella IgG and IgM detection and genotype determination was evaluated. However further validation of these methods in different settings is required before their routine use can be recommended.

Keywords: Dried blood spots; Oral fluid; Congenital rubella syndrome

1. Introduction

A world-wide vaccination campaign coordinated by the World Health Organization led to the eradication of smallpox with a last case in October 1977. Despite recurrent set-backs poliomyelitis is also well on the way to elimination. The transmission of indigenous measles virus (MV) has been interrupted in the Americas, and in many other countries where outbreaks result solely from imported cases. Recently, tremendous progress was made in Africa and Asia to further reduce measles-related mortality. The burden of congenital rubella has been largely underestimated in developing countries [1], but WHO initiatives have renewed interest in this debilitating condition [2–4]. In order to improve laboratory surveillance for measles and rubella, WHO has set up a world-wide Laboratory Network for Measles and Rubella [5]. In April 2005, the WHO Steering Committee on Research related to Measles and Rubella Vaccines met in New Delhi for its annual meeting to review and evaluate progress towards global control of these two diseases.

2. Measles

2.1. Public health significance of measles

WHO estimates that measles is responsible for 4% of the 6 million annual deaths in children under-five. Ninety-eight percent of these deaths occur in developing countries [6]. Thus, despite progress in global control, measles continues to be a serious condition and a leading cause of childhood death, particularly in developing countries. In 2004, WHO reported an estimated 76% coverage of measles containing vaccines (MCV) world-wide and 51% of countries reached ≥90% MCV coverage in all districts [7]. Most countries have provided a second opportunity for measles vaccination, either by introducing a routine two-dose schedule or by mass cam-

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paigns. With 30 million estimated annual cases [8], most of them unvaccinated, MCV is still under-utilized.

2.2. Progress in reducing measles mortality

In 2001, WHO and UNICEF developed a 5-year strategic plan to reduce measles global mortality by 50% in the year 2005, compared to 1999 levels [9]. In regions with established measles elimination goals, the objective was to achieve and maintain interruption of indigenous measles transmission. The plan targeted 45 priority countries with the following major strategies: (i) high routine vaccination coverage (≥90%) in every district; (ii) providing a second opportunity for measles immunization, mostly through supplementary immunization campaigns; (iii) improving surveillance; (iv) improving case management including vitamin A supplementation and antibiotic treatment if needed.

Global vaccination coverage increased from 71% in 1999 to 76% in 2004, but Africa and Southeast Asia lag behind with an increase from 50 to 66% and from 59 to 63%, respectively. In 2004, only nine of the 45 target countries offered no second opportunity for measles vaccination. In 2003, global measles mortality was estimated at 530.000 deaths, a 39% reduction from 1999 (Fig. 1). Indirect indicators suggested that the largest reduction in mortality was achieved in the WHO African Region [10], largely by implementing the above 4 components of the WHO–UNICEF strategy. Otten et al. [11] reported a decline in annual measles deaths of about 20% (90,043 of 454,000) as a result of supplementary immunization activities (SIA) in 19 African countries between 2000 and 2003.

2.3. Progress in eliminating indigenous measles transmission

Enormous progress towards measles elimination has been made in the Americas. In 1994, a goal was set to eliminate indigenous measles from the Western Hemisphere by 2000 [12]. Numbers of cases rapidly declined from 1990 to 1996. One year later, a large outbreak started in São Paulo, Brazil and spread to Argentina and Bolivia. Major vaccination efforts led by these countries reduced numbers of cases reported in the region to 1754 and 537 cases by 2000 and 2001, respectively. Few countries had continuing virus transmission [13] and not a single child has died from measles during the last 3 years. Molecular tools demonstrated that endemic measles transmission has been interrupted, but cases imported from other regions and residents who become infected abroad continue to be a problem [14,15]. These achievements were only possible thanks to the strong commitment from health authorities to implement and sustain the above components of measles control.

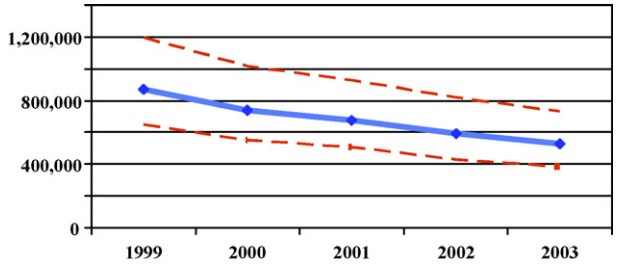

Fig. 1. Trends in estimated numbers of annual global measles deaths with uncertainty levels 1999–2003. Provisional data for 2003. Source: World Health Organization [59].

In the European Region and other countries, such as Australia, Mongolia, New Zealand, Philippines, the Pacific Island Nations and the Arab Gulf States, measles transmission has been interrupted or is at very low levels [16]. Also other regions have set elimination goals: the WHO European Region by 2007 and the Eastern Mediterranean Region by 2010. The Western Pacific Region plans to eliminate measles by 2012 [17].

2.4. Countries with indigenous measles transmission

Large countries, such as *Nigeria, India and Pakistan* continue to sustain large numbers of measles-related deaths (Dr. B.S. Hersh). In 2003, for instance, India reported more than 47,000 measles cases, while the 115 measles-related deaths are likely to be an underestimate. The country has used monovalent measles vaccine at 9 months of age since 1986; MMR is used only in the private sector. Reported coverage has been consistently high (>80%), but the estimated coverage is much lower (40–70%), and varies between states.

Other areas, such as *Niger* still report large outbreaks (Dr. C. Dubray). From November 2003 to June 2004, 11,073 cases were reported. Seventy-five percent of cases and 86% of deaths occurred in children under five. Attack rates were highest among the 6–9 month olds. Overall the case fatality rate (CFR) was 1.8%. CFR among under-fives and 12–35 month olds was as high as 2.7 and 4.6%, respectively. CFR was much higher (20%) among those admitted to hospital. The most prevalent complications were pneumonia (66%) and diarrhea (61%) followed by ear discharge (12.4%). This large outbreak was due to a failure to vaccinate, poor surveillance and an underestimation of CFR. In December following the outbreak, 94% of 9 months to 14-year-old children were vaccinated during a nationwide SIA. In the long term, routine vaccination coverage must be improved and a second opportunity for vaccination provided.

In *Nepal*, Dr. A. Joshi et al. conducted a community-based, retrospective study of a national, representative sample of 37 measles outbreaks (as defined by five or more epidemiologically linked measles cases) that occurred between March and August 2004 and were reported by health institutions throughout Nepal. Five thousand three hundred three measles cases with a median age of 6.4 years were reported; 25 and 60% of cases were 1–4 years and 5–15 years old, respectively. The crude CFR was 1.4%. The CFR was 8.9% for children <1 year, 2.9% for children 1–4 years, 0.5% for children 5–14

years, and 0.6% for children >14 years old. In response to these outbreaks, 9.5 million children were vaccinated.

Measles control is being integrated in many countries with other priority public health interventions, such as the distribution of insecticide-treated bed-nets, shown to reduce mortality due to malaria by one-third. Although there is a need to strengthen district-based immunization, current trends suggest that the goal of a 50% reduction of measles mortality by the end of 2005 compared to 1999 levels will have been met.

2.5. Estimating measles mortality

Given the weakness of disease surveillance and death registration in many parts of the world, routine reporting systems are not reliable enough to monitor measles mortality (Dr. L. Wolfson). A panel of experts met in January 2005 to discuss and advise WHO on methods for evaluating measles mortality reduction. It was recommended to use disease surveillance when data are reliable and mathematical models when data are limited. Despite their limitations, partially due to the periodic nature of measles outbreaks, mathematical models based on strong assumptions can capture important parameters, such as changes in vaccination coverage that influence mortality. Country-specific estimates of CFRs are a key driver in such models, however case-based surveillance with laboratory confirmation of suspected cases and documentation of cause-specific mortality should be implemented or improved in many countries. In 19 African countries, Otten et al. [11] reported an average decline in the number of reported measles cases of 91% resulting from supplementary immunization activities between 2000 and 2003, but whether or not the same reduction is seen in mortality is only assumed.

In this context, the SC recommended the following research priorities: (i) further work on the estimation of measles CFR in different settings and standardization of the methodology; (ii) careful evaluation of the strengths and weaknesses of estimations of the proportion of child deaths due to measles (i.e., proportional mortality); (iii) identification of factors that affect CFR within and between countries and (iv) evaluation of obstacles to high and sustained vaccination coverage.

2.6. Towards measles eradication?

As progress in controlling and eliminating measles in many parts of the world is encouraging, the pros and cons of global measles eradication were discussed (Dr. A.D. Osterhaus). Favorable factors include; (i) the absence of an animal reservoir; (ii) only rare persistent infections; (iii) the single virus serotype; (iv) the antigenic stability of the virus and (v) a safe and effective vaccine [18]. Challenges to measles eradication include (i) the impact of HIV endemicity on measles control; (ii) injection safety issues and the potential need for alternative routes of vaccination [19,20]; (iii) political and economic obstacles; (iv) appropriate strategies for different country-specific situations; (v) the high infectivity of the measles virus requiring a population immunity of 90–95% [21] and (vi) robust vaccine induced immunity. Dr. A.D. Osterhaus pointed out that vaccination may not necessarily be discontinued after eradication because of the risk of inadvertent reintroduction from a laboratory or from persistently infected individuals, and advertant reintroduction by bioterrorists, or animal morbilliviruses crossing the species barrier [22]. Furthermore, there are reports of non-specific benefits from receiving live-attenuated measles vaccine [23]. The benefits from eradication would include complete and sustained reduction of measles mortality and the absence of risk of virus transmission to pockets of seronegative individuals. A major risk of eradication would be a reduced vaccination compliance resulting possibly in the reintroduction of measles associated with high morbidity and mortality.

The SC recommended research in most of the above issues to obtain a better understanding of the implications of a measles eradication strategy. Thus, recommendations included the investigation of immunological correlates of protection, the characterization of long-term memory immunity, the development of standardized assays for measuring T and B cell immunity; the need to investigate cases putatively infected by genotype A viruses and the pathogenic potential of viruses isolated from these cases; and continued research on alternative candidate measles vaccines, which are non-replicating, effective at an earlier age and can be combined with other vaccines.

3. Rubella

3.1. Introducing rubella vaccine into a national program

WHO has recommended that developing countries without rubella vaccination programs should assess the burden of rubella and congenital rubella, in order to consider whether vaccination should be introduced [3]. This can be done either by surveillance, seroprevalence, retrospective studies or mathematical models. Guidelines for surveillance of rubella and CRS have been published [2]. Unlike for measles, there is no single strategy for rubella and CRS control. Two principal strategies may be considered: (1) selective vaccination of adolescent and adult females and (2) routine vaccination of all young children. The first strategy may prevent CRS, but does not control rubella. The second strategy can potentially control and eventually eliminate rubella and CRS, but carries the risk of increasing the average age of rubella cases thus resulting in increased instead of reduced numbers of CRS cases if vaccination coverage is insufficient [24]. The use of these strategies is dependent on many issues, such as infrastructure, goals of the program and funding [25].

In *South Africa* (Dr. L. Blumberg), rubella vaccination is not part of the national program and in the private sector uptake is very limited. Most rubella cases occurred in children less than 10 years of age and 18% of cases are between 10 and

14 years old. Even though fewer than 10% of the cases are among persons aged >14 years, there is a concern about CRS in newborns; however, there is no CRS surveillance system to directly measure this problem.

China (Dr. L. Gao) is also investigating how to identify the best strategy for rubella vaccination, but data on rubella and CRS prevalence is limited. Mathematical models based on current demographic trends, such as the one-child policy and declining birth rates, were used to compare different rubella vaccination strategies. Without vaccination CRS would increase 3-fold by 2050 as a result of the smaller birth cohorts. Childhood vaccination with <50% coverage would also increase CRS. Rubella and CRS could be eliminated if vaccine coverage is ≥80%. Routine vaccination of 12-year-old girls or mass campaigns in 15–40-year-old women would reduce CRS, but would have no effect on rubella incidence. Mass vaccination of 2–14 year olds would result in oscillating numbers of CRS cases with peaks above the no-vaccination level. These findings highlight the importance of changing demographic factors for the selection of the best rubella vaccination strategy.

Romania (Dr. A. Rafila) presented mathematical models based on epidemiological data of rubella of the last 45 years. As a result of decreasing birth rates, epidemic cycles increased from 5 to 10 years with most cases among children <15 years old. Between 1994 and 1998, >70% of cases were >15 years old; before and after this period <20% of cases were in this age group. In 2003, rubella vaccine was selectively administered nationwide to adolescent girls in 8th grade, then, in 2004 rubella-containing vaccine was added to the routine childhood schedule in addition to the adolescent female vaccination. The different models showed that (i) 80% vaccination coverage of children with a single dose during the second year of life would control rubella and eliminate CRS eventually. (ii) A catch-up campaign among children 2–14 years old would reduce the time to elimination, but would not be necessary for elimination. (iii) Vaccination of adolescent girls and young women would reduce CRS by 3/4 without affecting rubella incidence. (iv) Including older women of childbearing age would have no additional benefit and would only add to the costs.

While the robustness of mathematical models to estimate CRS incidence may be questioned, the tools for direct monitoring of CRS are limited and costly (e.g. detection of rubella virus RNA in extracted cataract material; systematic hearing tests).

3.2. Cost–benefit assessments of rubella vaccination

In *Bhutan* (Dr. T. Dorji) laboratory, diagnosis of rubella was introduced in 2003. Since then, rubella cases and several outbreaks have been confirmed. A retrospective study has documented a few clinical cases of CRS. Although rubella vaccination has been recommended, the best and most cost effective strategy and CRS surveillance are only being evaluated.

The *Republic of Maldives* (Dr. N. Ibrahim) does not vaccinate against rubella, but the disease became notifiable in 1999. Because of the relative isolation of the islands, the mean age of infection is 22 years, which is much higher than in most countries. Immunity among women of childbearing age is low, in particular outside of male and the outer islands (<45%). The costs of the 2000 outbreak and the lifetime costs of an infant with CRS defects have been evaluated. It was estimated that a mass campaign with MMR vaccine in children aged 6–14 years as well as male and female adults would result in savings of US$ 4.3 million.

Hinman et al. [26] reviewed 22 cost analyses of rubella vaccination including 10 from developing countries. The cost–benefit of rubella and MMR vaccine was evaluated each in five studies and their cost-effectiveness in two studies from developed countries. According to these studies, rubella vaccination programs have not only resulted in significant reduction of morbidity and mortality, but also in substantial cost savings [26]. The benefit–cost (B:C) ratio for a routine childhood program in three developed countries was estimated to be 5.8–11.1. In the Americas, the annual cost to treat an infant with CRS ranged from about US$ 1000 in Guyana (1997) and US$ 2291 in Panama (1989) to US$ 13,482 in Jamaica (1997). When compared with other vaccine-preventable diseases, such as *Haemophilus influenzae B* and Hepatitis B, rubella vaccination programs had a higher benefit–cost ratio. These studies are important in assisting governments to decide in which programs to invest limited resources.

3.3. Monitoring rubella control activities

After introducing rubella vaccination into the national program, monitoring of its impact is critical because insufficient coverage tends to increase the age at which susceptibles become infected. Thus, paradoxically the incidence of CRS may increase as a result of prevention. Countries that progress towards control of rubella, must be prepared to continuously adapt their vaccination strategy as exemplified below.

In *Italy* (Dr. M. Ciofi degli Atti), a national rubella vaccination program was established in 1972 targeting pre-adolescent girls. In the early 1990s, this schedule was modified to include a childhood dose of MMR at 15 months. In 1999, the age of the first dose was lowered to 12–15 months and a second dose of MMR vaccine recommended at either 5–6 years or 11–12 years of age. In 2003, the vaccination coverage among children aged 24 months was 77%. The epidemiology of rubella has changed since the introduction of rubella vaccines. Outbreaks occurred every 4–5 years, the last one in 1997. The mean age of infection increased from 9.5 years between 1976 and 1980 to 12.3 years between 1998 and 2003. Nevertheless, the incidence of rubella in women of childbearing age has decreased from 14.1 to 2.8 cases per 100,000 population, but there is no surveillance of CRS. In 2003, a national plan for elimination of measles and CRS by 2007 was established. Strategies include the target of 95%

vaccination coverage by 2 years of age; lowering the second dose of MMR to 5–6 years of age; conducting a catch-up campaign for unvaccinated children and adolescents and vaccinating susceptible women of childbearing age. Surveillance should be improved by mandatory notification of rubella during pregnancy and of congenital rubella.

In *Sri Lanka* (Dr. P. Palihawadana), the introduction of rubella vaccine was prompted by an increase in rubella cases and documented CRS cases. In 1996, rubella vaccine was first administered to adolescent girls and women of childbearing age. In 2001, two additional age cohorts (3 and 8 years of age) were added to the vaccination strategy. In 2001 through 2004, 72–82% of women of childbearing age were vaccinated. This is in agreement with results from a seroprevalence study conducted in 1999 in the Kalutara District (Sri Lanka; [27]). In children, coverage increased in 2003/2004 from 89 to 98%. Future plans include strengthening of laboratory confirmation of rubella and CRS as well as improving vaccination coverage by school-based programs.

Both countries have monitored the impact of their vaccination program by assessing their coverage data and have adapted their control strategies, which is particularly important for CRS control.

3.4. Surveillance of rubella and CRS

Studies from Vellore, South *India* (Dr. D. Brown) compared different methods for assessing the burden of CRS. Antenatal surveys in 1991 and 1998 showed that 8% of women were susceptible to rubella when 4 IU/ml was used as cut-off in a commercial enzyme immunoassay. Susceptibility was higher in the rural population than in urban women. Two age-stratified community surveys in rural and urban areas showed that the highest force of infection was in 5–9-year-old children. The estimated incidence of CRS ranged from 32–97 per 100,000 live births, but active hospital-based surveillance of CRS between 1996 and 2001 identified only 22 cases (0–9.4 cases per 100,000 live births), only 6% of predicted cases. Eleven (50%) of these cases had cataracts. The community survey was the most informative, but was also the most complex and costly. Rash/fever surveillance in pregnant women was recommended to identify children at risk of CRS. Another study in three eye-hospitals in South India showed that 11.7–20.8% of female hospital staff were susceptible to rubella [28].

Rubella IgM testing is the most convenient method for the confirmation of CRS, but commercially available tests have not been evaluated for this purpose [29] and some lack sensitivity [30]. This was also confirmed in studies in India. In addition, rubella IgM test kits are expensive and are not always available in developing countries. The SC recommended that available rubella IgM tests should be evaluated for diagnosis of CRS, using sera from children of different ages.

In *Morocco* (Dr. R. Ahmed), the CRS burden was assessed in a retrospective study [31]. Sixty-two cases of CRS by WHO case definition were identified from medical records, 148 from disability records and 15 among deaf schoolchildren. No laboratory confirmation was carried out. The yearly incidence of CRS was estimated to be 8.1–12.7 cases per 100,000 live births, a figure consistent with past estimates from the US and UK. The authors suggested that ophthalmology and cardiology departments may be best placed for finding cases of CRS. Retinal surveys in deaf schools were also convenient. The study concluded that rubella vaccine should be used in Morocco, where 15–34% of women at childbearing age are susceptible to rubella [reviewed by Bloom, 31].

Another recent report estimated the CRS burden in Iran in 1995/1996 by evaluating the proportion of children with sensorineural hearing loss attributable to rubella [32]. A case-control study tested 113 children in schools for the deaf and 112 controls aged 1–4 years for rubella antibodies. 19.5% cases and 8.9% controls had rubella antibodies. On the basis of these findings the proportion of cases with deafness attributable to rubella was estimated to be 12%, and CRS prevalence in Iran was calculated as 20 per 100,000 children. Iran has recently introduced childhood MMR vaccination and in a mass campaign about 32 million people aged 5–25 years were vaccinated with MR vaccine. A surveillance program should now be established to monitor the impact of vaccination, as recommended by WHO [2].

These reports show that identification of CRS cases is difficult and labor intensive. As the definition of CRS includes several defects, the specificity may be high but the sensitivity low.

3.5. Diagnostic value of RT-PCR for prenatal diagnosis of congenital rubella

The diagnosis of primary rubella infection is based on detection of specific IgM and/or rubella IgG seroconversion and rubella IgG avidity testing. The interpretation of results may be complicated by false-positivity, cross-reacting IgM or persistent rubella IgM [29,33]. When serological results are inconclusive, or primary infection occurs in the early second trimester or reinfection is confirmed in the first trimester, a prenatal diagnosis may be required to determine the risk of CRS for the fetus. RT-PCR can be used to detect RV RNA in amniotic fluid, chorionic villus biopsies and fetal blood, but few studies have been published [29]. Dr. L. Grangeot-Keros (Paris, France) reported results obtained from 110 amniotic fluids and 29 fetal blood samples from pregnant women with confirmed primary rubella infections. In this study, RT-PCR was a valuable tool for prenatal diagnosis, with a sensitivity of 83–95% and a specificity of 100% [34]. Detection of rubella IgM in fetal blood obtained by cordocentesis is also a valuable test for prenatal diagnosis, but the collection of fetal blood is technically more demanding ([29]; Grangeot-Keros, personal communication). It is important that specimens are transported at 4 °C and are collected no earlier than 7 weeks after infection (reviewed by Best and Enders [29]).

4. Genotyping of measles and rubella

Laboratory surveillance is a key component of measles and rubella control programs. It includes serological confirmation of suspected cases and genetic characterization of endemic/epidemic viruses. Genotyping is useful for molecular epidemiology and surveillance and allows tracking of virus transmission, e.g. to demonstrate elimination of indigenous MV and RV from the USA [35,36]. Genetic characterization of MV has been proven to be a valuable tool for measuring the effectiveness of measles control programs [37]. In a given geographic region, a genotype is considered endemic if it is consistently found in the same region over an extended time period. If different genotypes are associated with limited outbreaks and/or sporadic cases, these are likely to result from multiple importations of viruses into the region rather than from circulating endemic viruses [38,39]. Both for MV [40] and RV [41], WHO recommends a standardized nomenclature.

4.1. Measles virus

Genotypes are determined by phylogenetic analysis of the 450 C-terminal nucleotides of the nucleoprotein gene and/or of the entire hemagglutinin gene [42]. There are currently 23 recognized genotypes (A, B1–3, C1–2, D1–10, E, F, G1–3, H1–2; 39), most of which are actively circulating in more or less confined geographic regions with possible long distance importations [39,44]. The SC recommended to further improve molecular tools for detection and characterization of MV and to continue the study of virus transmission patterns via molecular epidemiology, to help monitor measles control programs.

4.2. Rubella virus

Various regions of the E1 gene have been proposed by different authors for genetic characterization. A window of 739 nucleotides (nt 8731–9469) is recommended for routine molecular analysis [41]. Two major phylogenetic groups of RV designated clades 1 and 2 have been described, differing by 8–10% of their nucleotides. Seven genotypes designated with upper-case letters (1B, 1C, 1D, 1E, 1F, 2A and 2B) have been accepted. Reference viruses are available for these genotypes. Three provisional genotypes (1a, 1g and 2c) have also been described. Clade 1 viruses currently exist world-wide, while clade 2 viruses have been found predominantly in Asia, with occasional isolates from Europe [41]. Clade 2 viruses have not been detected in the Western Hemisphere (Fig. 2).

Dr. W. Xu (*China*) reported on the epidemiology and circulating genotypes of rubella in China. Locally produced BRD-2 rubella vaccine was approved by the Chinese regulatory authorities in 1998 and is now used routinely in many provinces. Sentinel surveillance sites reported a major peak in rubella incidence in 1993 (98 cases/100,000) and a few outbreaks in Shandong province in 2001. Sequencing of 601 bp of the E1 coding region (nt 8869–9469) of 42 isolates collected between 1999 and 2003 from Anhui, Henan and Shandong provinces, identified genotypes 1E, 1F, 2A and 2B. In 2001, rubella cases caused by both genotypes 1F and 2A occurred within 100 km from each other in Shandong province. The heterogeneity of RV is much greater than that of measles viruses isolated between 1993 and 2005 in China suggesting that measles control is a ahead of rubella control.

The SC recommended that genetic analysis and isolation of RV should be continued, as the circulating viruses from many countries have not been characterized. Specimens for

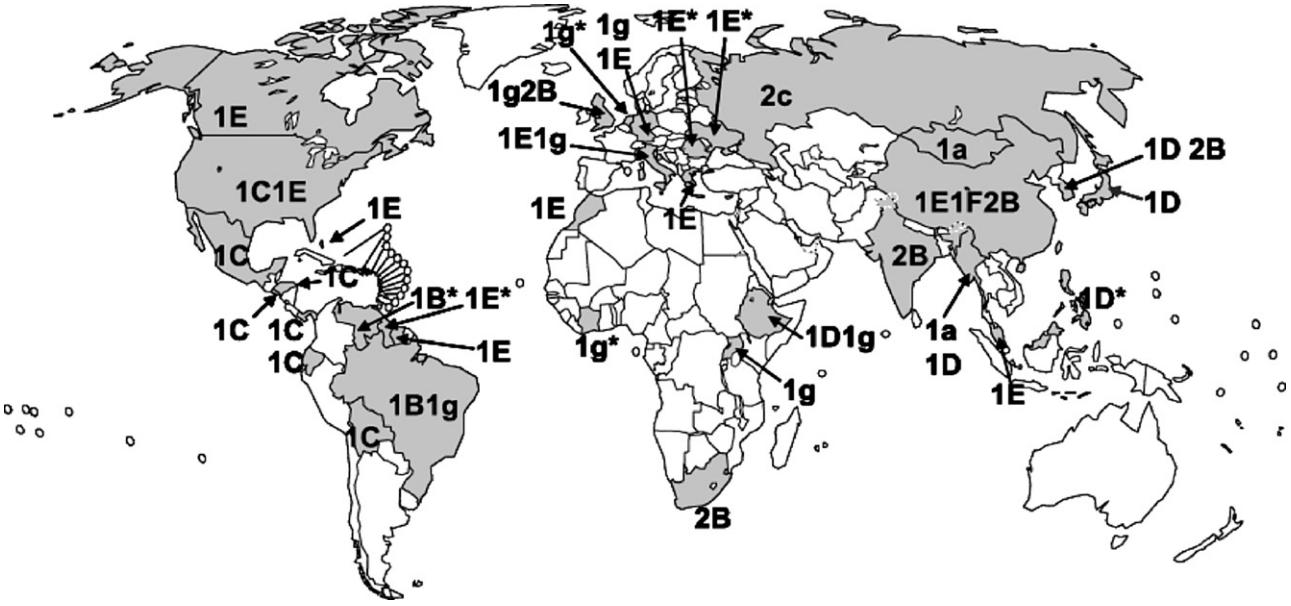

Fig. 2. Geographic distribution of genotypes of rubella virus (1995–2005). Clade 1 viruses currently exist world-wide. Clade 2 viruses have not been found circulating in the Western Hemisphere.

virus isolation and/or sequencing should be obtained at the same time as serum for serological diagnosis and sent to the local WHO network laboratory [5].

5. Alternative sampling for diagnosis of measles and rubella/CRS

Case confirmation relies on IgM testing using validated ELISA tests (e.g. Dade Behring) for measles. Serum is considered the preferred sample for case confirmation but less invasive sampling techniques are being evaluated [5]. Dried blood spots (DBS) and oral fluid (OF) have both been confirmed to be suitable for IgM testing [45,46] and for RT-PCR and sequencing [46]. DBS are collected by finger prick onto filter paper (Whatman 903, Schleicher & Schuell), which is easier and considered less invasive than venupuncture [47]. As DBS are not considered "Dangerous Goods" for transport purposes, packaging and transportation to the laboratory is also simplified. DBS are reconstituted in the laboratory and tested for IgM by ELISA [45]. OF collection is acceptable to patients, particularly children, as it is non-invasive and painless. OF consists of gingival crevicular fluid; therefore more sensitive assays are required [48]. Best results were obtained when OF was collected using a special device [49] and tested for IgM by capture EIA (e.g. Microimmune, Brentford, UK).

The SC recommended further optimization of elution techniques and further development of RT-PCR for use with DBS.

5.1. Measles

DBS tested with a modified Behring ELISA has a sensitivity of 97–100% and a specificity of 91–100% for measles IgM, OF using Oracol and the Microimmune IgM assay has a sensitivity of 96–99% and a specificity of 84–100%.

Typically, genetic typing relies on RT-PCR of viral RNA from virus cultures or directly from peripheral blood lymphocytes, urine and nasopharyngeal swaps. For virus isolation Vero-SLAM cells have been recommended to replace B95a cells, which actively produce Epstein-Barr-Virus [43]. OF can also be used for virus isolation and RNA detection by PCR [50]. In DBS, the lower sensitivity for RNA detection can be partially offset by a larger amount of DBS and a nested PCR [51].

OF was also used to evaluate seroconversion after vaccination and to detect and genotype measles virus in Ethiopia [52]. In this setting, OF provided a convenient, non-invasive sample for both IgG-serology and virus surveillance. As there was some concern about the sensitivity of IgG detection in oral fluid from vaccinees, which usually have lower IgG levels than late convalescents, further evaluation of the methodology in different settings is needed.

The Subcommittee concluded that regions without measles control, with only minimal laboratory support and without adequate access to expertise/supplies for conventional specimen collection, may benefit from the use of DBS or OF. Whenever possible, conventional sampling methods should be performed on a few cases of an outbreak. Regions with controlled measles but periodic outbreaks could initiate DBS or OF sampling in parallel with conventional methods. Regions in elimination phase with few outbreaks would not generally benefit from alternative sampling methods and should be evaluated on a case-by-case basis. Accordingly, the SC has asked WHO to continue field trials of these techniques in different stages of measles control.

5.2. Rubella

A commercial test for rubella IgM in OF is under development, and field trials are required. Dried blood spots can be used to detect rubella antibodies [53,54]. OF can be used to detect rubella IgM in both postnatally acquired rubella and CRS, but is not suitable for rubella IgG detection in adults [55–57]. RNA can be extracted and used for detection of RV by RT-PCR [58]. OF has been found to be better for nucleic acid extraction than serum or blood due to lack of PCR inhibitors.

The SC considers the preliminary studies as promising and recommends further evaluation of OF. WHO should produce protocols for use of these alternative sampling methods, including their limitations. Further research should also determine whether RV sequences can be reliably obtained from alternative specimens, such as serum, DBS or OF.

6. Concluding remarks

Following the guidelines of the Strategic plan for measles control 2001–2005 developed by WHO and UNICEF, worldwide measles morbidity and mortality have been significantly reduced. The Steering Committee has developed research guidelines to support future measles reduction strategies. These include the evaluation of population immunity, outbreak investigation, design of vaccination strategies as well as the optimization of surveillance activities including the implementation and validation of new diagnostic tools. The surveillance and control of rubella and CRS lags behind measles control. Therefore, the guidelines concerning rubella and CRS focused on optimizing the tools to assess the burden of disease, to identify the best vaccination strategy for a given setting, and to improve diagnostic methods, particularly for the diagnosis of CRS.

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