World Health Organization

REGIONAL OFFICE FOR Europe

MEETING REPORT

MEETINGS OF THE MEASLES / RUBELLA LABORATORY NETWORK IN THE WHO EUROPEAN REGION

13-16 NOVEMBER 2018, COPENHAGEN - DENMARK

Keywords

DISEASE ELIMINATION
SURVEILLANCE
EPIDEMIOLOGY
IMMUNITY
LABORATORIES
ACCREDITATION
VERIFICATION
MEASLES
RUBELLA

Address requests about publications of the WHO Regional Office for Europe to:

Publications
WHO Regional Office for Europe
Marmorvej 51
DK - 2100 Copenhagen Ø, Denmark

Alternatively, complete an online request form for documentation, health information, or for permission to quote or translate, on the Regional Office website (http://www.euro.who.int/pubrequest).

© World Health Organization 2020

All rights reserved. The Regional Office for Europe of the World Health Organization welcomes requests for permission to reproduce or translate its publications, in part or in full.

The designations employed and the presentation of the material in this publication 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 or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either express or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use. The views expressed by authors, editors, or expert groups do not necessarily represent the decisions or the stated policy of the World Health Organization.

Table of contents

Abbreviations

AFRO	World Health Organization Regional Office for Africa
AMRO	World Health Organization Regional Office for Americas
ASU	Annual status update on measles and rubella elimination
ASCII	American Standard Code for Information Interchange (a character encoding standard for electronic communication)
BEAST	Bayesian evolutionary analysis sampling trees
BLAST	Basic Local Alignment Search Tool
CDC	United States Centers for Disease Control and Prevention
CIS	Commonwealth of Independent States
CISID	Centralized Information System for Infectious Diseases
CRI	Congenital rubella infection
CRS	Congenital rubella syndrome
ECDC	The European Centre for Disease Prevention and Control
EIA	enzyme immunoassay
ELISA	Enzyme linked immunosorbent assay
EMRO	World Health Organization Regional Office for Eastern Mediterranean
ES	enhanced (active) surveillance
EQA	External Quality Assessment
EVAP	European Vaccine Action Plan
EU	European Union
EUR	European region
EURO	European regional office
GSL	Global specialized laboratory
HHV-6	Human herpesvirus 6
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LabNet	Laboratory network
MCV	Measles-containing vaccine
MF-NCR	Non-coding region located between the matrix and fusion protein genes of measles virus genome
MeaNS	Measles Nucleotide Surveillance
MeV	Measles virus
mEQA	Molecular external quality assessment
MIBE	Measles inclusion-body encephalitis
MoH	Ministry of health
MR	Measles/Rubella
MRLDMS	Measles and rubella laboratory data management system
NGS	Next-generation sequencing
NIBSC	National Institute for Biological Standards and Control
NIS	Newly independent states
NL	National laboratory

NP	nucleoprotein
NRL	National reference laboratory
NVC	National verification committee
N-450	Measles virus genotyping region: 450 nucleotides C-terminus of nucleoprotein gene
nt	nucleotide
OF	Oral fluids
PAHO	Pan-American Health Organization
PCR	Polymerase chain reaction
PHE	Public Health England
PRN	Plaque reduction neutralization
PP	Proficiency panel
PT	Proficiency test
RCV	Rubella containing vaccine
RF	Russian Federation
RKI	Robert Koch Institute
RNA	Ribonucleic acid
RRL	Regional reference laboratory
RubeNS	Rubella Nucleotide Surveillance
RuV	Rubella virus
RVC	Regional Verification Commission
SAGE	The Strategic Advisory Group of Experts on Immunization
SEARO	World Health Organization Regional Office for South-East Asia
SIA	Supplemental immunization activities
SNL	Sub-national laboratory
SSPE	Subacute sclerosing panencephalitis
Tessy	The European Surveillance System
UK	United Kingdom of Great Britain and Northern Ireland
USA	United States of America
VIDRL	Victorian Infectious Diseases Reference Laboratory
VPI	Vaccine preventable diseases and immunization
WGS	Whole genome sequence
WGS-t	Whole genome sequence excluding 3’ and 5’ termini
WPRO	World Health Organization Regional Office for Western Pacific

EXECUTIVE SUMMARY

On 13–15 and 14–16 November 2018, two partially overlapping meetings of the WHO European Regional Measles/Rubella Laboratory Network (MR LabNet) took place in Copenhagen, Denmark. The first meeting was attended by representatives from laboratories in western and central European countries; the second was attended by representatives from laboratories in the Russian Federation and Newly Independent States. In both meetings participants were updated on progress and developments related to the MR LabNet and disease elimination programmes at regional and global levels, new molecular testing protocols, revised laboratory algorithms in connection with the new WHO Measles Rubella Laboratory Manual, serological testing kit comparison and validation and molecular external quality assessment (EQA). Also discussed were laboratory accreditation issues, the laboratory contribution to verification, reporting to WHO and eLearning tool development. A skills-strengthening session on viral sequence analysis and management, including reporting to the WHO Nucleotide Surveillance databases, was provided.

1. Introduction

The European Regional MR LabNet was established in 2002 to support high-quality surveillance for measles and rubella and monitor progress in the disease elimination programme. Network laboratories have an increasingly important role in confirming suspected measles, rubella, and congenital rubella syndrome (CRS) cases and monitoring viral genotypes when cases occur. With regional efforts focused on accelerating the elimination of measles and rubella, laboratories are making substantial contributions to the elimination and verification processes, but many are experiencing increased workloads. Two partially overlapping meetings of the MR LabNet, one for laboratories in western and central European countries, the other for laboratories in the Russian Federation and Newly Independent States, took place in Copenhagen, Denmark from 13th to the 16th of November 2018.

2. Sessions of the meeting

Session 1A – Training on Measles and Rubella viral sequences management

Chair: Dr Sabine Santibanez (RKI, Berlin)

Virus sequence data management is increasingly important in the disease elimination and verification phases. The web-accessible measles nucleotide surveillance database (MeaNS) and rubella nucleotide surveillance database (RubeNS), developed and maintained for the MR LabNet by Public Health England (PHE), are essential tools in the monitoring of measles and rubella transmission and in providing evidence for the elimination verification process.

1A.1. Measles and rubella viral sequence management

Dr Kevin Brown, PHE, United Kingdom, provided an overview of the measles and rubella virus genome structures, methods used for identifying and distinguishing virus genotypes and changes in observed genotype distribution associated with the elimination programme.

Measles virus (MeV) genotyping is currently based on sequencing the highly diverse N-450 coding region and comparison to the sequences of assigned genotype reference strains. Known MeV strains belong to 1 of 8 clades (A-H), further divided into 24 genotypes (A, B1-B3, C1-C2, D1-D11, E, F, G1-G3, H1-H2). A number of genotypes (B1, C1, D1, E, F, G1) appear to have been eliminated as they have not been detected

for many years. Viruses belonging to genotypes C2, D2, D3, D5, D6, D7, D10, G2, H2 have become rare and appear on the verge of elimination. Data from MeaNS suggests that only 5 MeV genotypes are currently circulating globally (B3, D4, D8, D9, H1), and only 2 genotypes commonly circulate in Europe (B3 and D8). Decreasing genotype diversity is making it increasingly difficult to distinguish endemic from imported virus strains based on genotype data alone. A study conducted by PHE in 2012 suggested that sub-genotyping, based on the variation in measles sequence was unlikely to be of great benefit, but that identifying identical N-450 sequences may be usefully for tracking outbreaks. Virus strains with identical N-450 sequences, particularly when associated with outbreaks, become labelled as ***named strains***. Sequences of named strains should be made available in accessible databases, i.e. GenBank, so they can be used globally. With increasing dependence on genome sequencing it is essential that all participating laboratories establish a high level of proficiency in sequence analysis. The CDC protocol for amplifying the N-450 region and sequencing primers is available in the new WHO Laboratory Manual (Annex 7.1) and additional methods are being published. Any laboratories in doubt over the correct method to use or implementation details should contact their RRL. Sequences obtained should to be compared to sequences of WHO reference strains, which can be downloaded from the MeaNS site. It is expected that all MeV sequences will be submitted to MeaNS in a timely manner. Detailed information on how to submit sequences to MeaNS was provided at the 2016 MR Regional Labnet meeting in Budva, Montenegro. Sequences are stored using the WHO name, making it essential to name strains correctly and consistently before submission, as this field cannot be changed after submission. The format of the WHO name is as follows:

Mvi or MVs/city.ISO/wk.year/number [genotype] (special)

Example: MVs/London.GBR/3.12/2 [D4]

MVi - sequence derived from cultured virus isolate
MVs - sequence directly from clinical material
city - city/state or province where the case occurred
ISO - 3 letter ISO country code
wk.year - epi week (week 1 is first Monday of year) and year
number - sequence number, if >1 sequence the same week and location (from 2 upwards)
genotype - optional (MeaNS will give genotype anyway)
special - MIBE or SSPE; the original version of MeaNS did allow to put VAC here, but vaccine strains should not be submitted to MeaNS, it's supposed to be a wild-type sequences database.

Rubella virus genotyping is based on sequencing the 739 nt window of the E1 gene followed by comparison to reference strains. The procedure is similar to that used for measles but laboratories need to generate two amplicons to derive the sequence. The naming convention for rubella is the same as for measles. Far fewer sequences are available for rubella and it is important to add more sequences to the database to permit molecular epidemiological analysis. Countries where rubella continues to circulate are strongly encouraged to collect samples and send them to their RRL or GSL if they are unable to sequence them. A number of different methods have been published for generation of amplicons and CDC has published a method which is described in the new WHO Laboratory Manual (Annex 7.1). A number of other published

methods are available, including single amplicon methods. If methods other than that of CDC are used it is essential to ensure that all of E739 is included in the sequence analysis.

Discussion

- Concerns were raised over Protecting Individuals Rights in the countries with few measles cases and whether a patient could be recognized in the MeaNS database from the name of the sequence. It was stressed that MeaNS is not a public database, Protecting Individuals Rights is adequately addressed.
- MeaNS provides a powerful tool for aligning a genotype, but is not always fully understood by users. It is essential that MR LabNet staff using the tool should be able to understand the process.
- Concerns have been raised over errors introduced by reverse transcription or associated with the polymerase in the early stages of amplification. However, in practice these errors are now extremely unusual and if suspicions are raised the genome can easily be re-sequence. It is known that nucleotide differences are introduced into MeV at a low rate, and observation of a unique sequence, when there is confidence in the proficiency of sequencing, is not an indication for delaying submission to MeaNS.
- The likelihood of detecting new sequence variants in countries such as Germany, with a high incidence rate for measles, is reasonably high. These variants can be confirmed through the detection of chains of transmission.
- It is possible to occasionally detect mixed nucleotides in samples from measles patients. In the UK the mixture is confirmed by first viewing in both forward and reverse directions, and secondly either by resequencing from the beginning or by confirming the same mixture in a different sample from the patient. MeaNS currently does not allow entry of mixed sequences, but the recommendation is to select one of the sequences and add a note to MeaNS identifying it as a mixed base.
- There is a danger of over-interpreting the information provided in MeaNS and it is essential that epidemiological information is collected and considered in conjunction with the sequence data before a decision can be made on whether a case is considered endemic or import-related. Even in the countries with intensive surveillance, cases are clearly being missed as sequences are sometimes identified in cases with no epidemiological linkage to other cases. In addition, the intensity of molecular surveillance is not particularly high in countries in other WHO regions, so in Germany, for example, new variants are sometimes detected in cases imported from South East Asia, but these sequences are not found in MeaNS.

1A.2. Practical exercises on viral sequences management

Dr Kevin Brown facilitated a practical sequence analysis exercise for measles and rubella using GeneStudio Pro software.

Discussion

- It is not recommended to use the vaccine as the positive control. Positive controls should be viruses that are not circulating and it is strongly recommended to use an extinct strain of genotype A. This permits the distinction to be made between the positive control and the vaccine strain in case of

laboratory contamination. NIBSC has a quantity of measles positive controls, deposited there for use by the MR LabNet laboratories. The only cost to obtain a control, is the cost of shipping.

- If the laboratories have software that allows viewing of chromatograms in both forward and reverse directions at the same time, they should continue to use these. If laboratories currently use MEGA or BioEdit software that do not allow this, it is recommended to start using GeneStudio Pro (free) or request the host institution to obtain it commercially.

Session 2A – Case studies

2A.1. Group work on 3 case studies (presentation of cases and discussion)

Chair: Dr Kevin Brown (GSL, London)

Clinical and epidemiological data, and results of laboratory investigation were presented by Dr Sabine Santibanez for three measles cases that occurred in Germany, and meeting participants were requested to provide interpretations of the cases.

Discussion

- Concerns were raised over diagnosis of reinfection cases (secondary vaccine failure) using serological methods alone if the IgM result is negative. In these cases, it was recommended to use capture assays, which can be more sensitive than standard assays. It is also possible to confirm reinfection by a rise in IgG titre in sequential samples taken 1-2 weeks apart.
- Concerns were also raised over potential negative effects on public confidence in vaccination when results of vaccine failures or reinfection are published. The number of these cases detected has increased recently due to very high vaccine coverage and increased surveillance for measles in most countries. There is strong evidence that cases of reinfection are significantly less infectious that cases with primary infection. As progress is made towards elimination of measles laboratories should expect the number of detected cases of reinfection to rise, know how to interpret these results and be prepared to provide appropriate guidance to the programme. Reporting of cases of reinfection is being addressed by the WHO SAGE Measles and Rubella Working Group, and guidance will be provided.
- Laboratories should be using the new testing algorithms available in the 3rd edition of the WHO Laboratory Manual. The algorithms are also available in the new VPD Surveillance Standards document released in 2018.
- There is a risk that private laboratories and SNLs conducting diagnostic testing are discarding cases that are IgM negative and IgG positive. Usually only IgM positive samples are referred to the NL and if IgM negative samples are referred there is often a lack of supporting information.

Dr Judith Hubschen presented information on hypothetical outbreaks of rash and fever and participants were requested to discuss any additional laboratory testing required, interpretation of additional laboratory results, and potential guidance to give to the programme

Discussion

- Oral fluids (OF) can be tested for measles IgM and for rubella IgM, and also for measles IgG. It is sometimes possible to culture MeV, but extremely difficult, especially when IgG is present and also

because OF is usually contaminated with mouth flora. It is even more difficult to culture RuV from OF.

Session 3A – Q&A: Accreditation issues

Chair: Kevin Brown (GSL, London)

3A.1. Update on accreditation check-list

Dr Myriam Ben Mamou welcomed participants from the NLs of Switzerland and Montenegro who were attending the meeting for the first time. The WHO measles and rubella laboratory accreditation programme was established in 2002 and is based on 2 pillars: desk reviews and external audits of laboratories within the network using standard accreditation checklists. The accreditation process also includes annual proficiency testing of serological and molecular capacities of network laboratories. There are ten accreditation criteria, divided into four blocks: reporting, EQA/IQC, workload, and on-site review.

Discussion

- Laboratories that conduct RT-PCR before serology have requested a longer IgM results reporting period to meet the accreditation criteria. The accreditation process takes an individual approach, attempting to incorporate the differences between participating laboratories, but it is not possible in the check-list to fully accommodate every participating laboratory.
- The monthly reporting of IgM results to CISID requires laboratories to submit the number of tests reported within 7 days and in 7-14 days, but the annual accreditation check-list requires laboratories to provide the number of tests reported within 4 days; it would be easier for laboratories to make only one calculation.
- Laboratories conducting few molecular tests for measles and rubella were not aware of the new criterion to have at least 2 tests conducted per laboratory each quarter.
- Several laboratories do not have in-house rubella controls for serology, which reduces their score on the PT panel. There are commercial companies that supply in-house rubella controls; however, it has been noted that commercial controls may give very high OD values, and the sensitivity of the immunoassay cannot be checked.
- A new accreditation criterion to report results to SNLs within 14 days was introduced for countries with SNLs that are officially part of the WHO laboratory network. If NLs of other countries receive samples for confirmatory testing purposes from their national network, they should also apply this criterion.

3A.2. Update on EQAs: serology, molecular, IQC

Dr Myriam Ben Mamou, WHO regional office for Europe, provided an update on EQAs.

Serology PT

There is a public website for the serology PT[1] with general instructions and result submission instructions. Obtaining the correct result and interpreting it correctly for the 20 panel samples accounts for 75% of the final score; the remaining 25% of the score is associated with completeness of information and timeliness

[1] http://www.vidrl.org.au/laboratories/measles-reference/measles-rubella-proficiency-panel/

of reporting. There are still some laboratories that correctly identify the samples but fail to achieve a 100% result because of a failure to submit all required information in a timely manner.

Molecular EQA

In 2015/17 molecular EQA for laboratories in the European Region were conducted through INSTAND. Overall there was an improvement in molecular performance during the three rounds of mEQA conducted from 2015 to 2017, with both measles and rubella sequencing performance having improved. However, the quality of detection of measles and rubella by RT-PCR decreased from 100% to 97.1% for measles and from 96.7% to 90.6% for rubella. The fourth round of mEQA, organized by CDC, included a total of 37 laboratories, with 37 laboratories being assessed for measles RT-PCR, 35 laboratories for rubella RT-PCR; 31 laboratories for measles sequencing and 19 laboratories for rubella sequencing. Laboratories in Georgia, Montenegro and Macedonia will participate in the mEQA for the first time in 2018.

Discussion

- Concerns were raised over shipping of PT panels. It is not possible for RRLs to ship serology PT and mEQA panels to countries at different times. Given the size and complexity of the network is only feasible for RRLs to make only one shipment per year.

Session 4 – Global and regional updates

Chair: Kevin Brown (GSL, London)

Welcome and opening remarks

The participants were welcomed on behalf of the Regional Director by Dr Siddhartha Datta, Programme Manager, Vaccine Preventable Diseases and Immunization. The increasing number of new members of the MR LabNet demonstrates how much Member States value the role of laboratories in measles and rubella elimination. WHO highly appreciates the contribution and dedication of all 73 members of EUR MR LabNet, together with the technical and financial support that RRLs in Berlin, Luxembourg and Moscow, as well as the GSL in London, provide to the network. In September the midterm report of the EUR Vaccine Action Plan was presented to all member states at the 68th Session of the WHO Regional Committee for Europe in Rome, and the role and contribution of the laboratories was acknowledged.

4.1. Regional measles and rubella programme update

Dr Patrick O'Connor, WHO regional office for Europe, provided a programme update. Whilst the annual Regional cases counts for measles and rubella have declined very dramatically since 1974, and vaccine coverage has risen to impressive levels, cases continue to occur. Coverage with a first dose of measles-containing vaccine (MCV1) has remained stable at approximately 93-95%, and coverage with a second dose of measles-containing vaccine (MCV2) has been maintained at 88-91% over the past several years. After years of decline the number of measles cases in the Region has shown an upsurge in 2018, due predominantly to immunization gaps at subnational level. Of the cases confirmed in 2018 approximately 25% were <5 years of age, 54% were aged 5 to 29 years, and 20% were aged 30 and above. While the

majority of cases in 2018 have been reported from a small number of countries, the entire Region has been affected by the upsurge in cases.

The number of reported rubella cases in the Region has remained relatively static since 2013, but a significant number of reported cases are not laboratory confirmed, and in 2017 only 17% of reported cases had laboratory confirmation. Considerable effort is required to increase the level of laboratory confirmation to exclude the possibility of over-reporting. In 2017 Poland reported 496 rubella cases (69.6% of all reported cases in the region), and none of these were laboratory confirmed. Germany reported 11 laboratory-confirmed, 7 epi-linked and 54 clinically-compatible cases.

Discussion

- A large number of measles cases in 2018 were reported with unknown vaccination status, particularly cases in older age groups. In many countries there are no records available for vaccination of older age groups. Case investigation usually only takes written records of vaccination status into account, not patient recall.
- In the majority of countries experiencing large measles outbreaks a significant proportion of cases are zero dose cases.
- The upsurge in cases and occurrence of several large outbreaks of measles points to the importance of subnational or community level data where we have large pockets within countries that perform well at the national level, but do not perform at subnational level. This is a very heterogeneous region, and the reasons for not being vaccinated are complex, including vaccine hesitancy, vaccine supply issues and economic and social migration.

4.2. WHO global programme and LabNet update

Dr Mick Mulders, WHO HQ, Geneva, provided a global programme update. In the face of dramatically improved surveillance, the number of measles cases reported worldwide markedly decreased from the introduction of measles vaccines until 2007. While there has been only a modest reduction in the global measles case load since 2007, there has been a reduction in measles deaths since 2000, with an estimated 20.4 million deaths prevented by measles vaccination. Despite this impressive reduction, it is still short of the 2015 target of a 95% reduction. Importantly, for both cases and deaths, progress has levelled off over the past 10 years and all three 2015 control targets have not been met. In 2017 global vaccine coverage with MCV1 was around 85%, while coverage with MCV2 was 67%. In 2017, only 118 (61%) countries reached the target of ≥90% coverage with MCV1; and 45 (23%) countries achieved ≥90% national MCV1 coverage with ≥80% in every district. As of 2017, there are still 27 countries out of a total of 194 that have not yet introduced MCV2 into their routine immunization schedules.

Reported measles cases by WHO Region from 2014 to 2017 shows dramatic reduction of cases in the Western Pacific Region (WPR) mostly due to increased vaccination efforts in China. Some reduction has also been seen in the South East Asian Region (SEAR) accompanied by significant improvement of surveillance in India. The Eastern Mediterranean (EMR) and African (AFR) Regions again experienced a high incidence of measles. There has been a dramatic increase in measles cases in the Americas Region (AMR) since October 2018. AMR has lost its verified endemic measles-free status because of high measles incidence in Venezuela, Brazil and Columbia.

There has been an increase in the number of countries that have introduced RCV into their routine immunization schedules, rising to 84% in 2017. Global RCV coverage is now estimated to be 52%. A significant reason for the low global coverage is that several Member States, mainly in Sub-Saharan Africa and the Southeast Asian region, have yet to introduce rubella vaccine and have no known plans for introduction. Data on rubella incidence is limited and laboratory data is largely based on results of testing negative suspected-measles cases for rubella, particularly in AFR. There continues to be significant underreporting of rubella, with several countries that experience outbreaks failing to report cases to WHO in a timely manner. Globally a minority of Member States have adequate surveillance sensitivity for measles and rubella as assessed by the suspected case discard rate.

The GMRLN is the largest globally coordinated laboratory network, currently comprised of 714 laboratories, and continuing to grow. There are 14 RRLs and WHO HQ is in the process of nominating a 4th GSL in Beijing, China. In 2017 the laboratory workload included approximately 162,000 specimens tested for measles (28% positive) and approximately 128,000 specimens tested for rubella (10% positive). Data as of October 2018 already indicates that in 2018 the number of measles and rubella specimens tested will exceeded that of 2017.

The most common circulating measles strains in the last 12 months belong to genotypes B3 and D8, with some H1 continuing in China and neighboring countries. Several countries, particularly in ARF and EMR, are still not conducting genotyping on laboratory-confirmed measles cases or are reluctant to share their genotype data with WHO. The overall picture from the past 12 months is less positive for rubella with only a few countries in EUR and a few in Asia that are reporting rubella virus sequences. Japan has shared RuV sequences from the outbreak there (both 1E and 2B genotypes), but does not report other data on rubella cases to WHO.

WHO has 10 Working Groups to direct the GMRLN and provide guidance to the countries, including a group responsible for developing the 3rd edition of the Measles and Rubella Laboratory Manual. New guiding documents have recently been published, or are expected soon, including: VPD Surveillance Standards; Serosurvey guidelines; and new guiding document on NGS.

Critical issues to be addressed include the sharing of sequence data in a timely and accurate manner; correct use of named strains; improved collection of specimens for virus genotyping; improved linkage of laboratory and epidemiologic; use of standardized testing algorithm for case classification particularly for reinfection cases in elimination settings; sharing sera for the EQA program; standardized methods for MF and WG sequencing. Challenges faced by the GMRLN include the assessment of existing immunoassays that can replace the Siemens kit used in the GMRLN; cross border sample transportation, particularly in AFR; sustainable funding support; competing priorities, particularly dealing with emergencies; and, maintaining laboratory competence in the face of staff turnover.

Discussion

- With the limited sequence diversity that is currently seen, it is almost impossible to determine where the virus associated with the recent measles outbreaks in the Americas originally came from. In many respects it is not important to know where the virus came from, it is important to maintain population immunity to levels high enough to prevent transmission of any imported virus.

- Concerns were raised over potential inaccuracies in the estimation of measles mortality and incidence. The models used by WHO are based on surveillance data collected over many years, but the data is far from comprehensive. A recent publication by Dr Minal K. Patel estimated that only 3% of measles cases are being reported. Measles mortality in EUR is very low, but in AFR, particularly in immunocompromised individuals and those with secondary infections, mortality due to measles is much higher.
- It is now generally accepted that only a small number of measles genotypes are circulating globally, despite the lack of genotyping data from several large countries. Because of the transmissibility of measles, it is assumed that representative strains from all outbreaks will eventually be detected in countries that have genotyping capacity and their results reported to MeaNS.

4.3. Regional measles and rubella LabNet update

Dr Myriam Ben Mamou, Regional Laboratory Coordinator, provided an update on the Regional measles and rubella situations. The Regional MR LabNet has a 4-level structure with one GSL (PHE, London, UK), 3 RRLs (RKI, Berlin, Germany; The Gabrichevsky Institute, Moscow, Russian Federation; the Institute of Health, Luxembourg), 50 NLs in 50 Member States, and 19 official WHO SNLs. In addition, some countries (Italy, Kazakhstan and Romania) have established their own sub-national networks of MR labs. Laboratory performance is monitored through an annual laboratory accreditation programme based on annual desk review using an accreditation check-list. Periodically on-site accreditation visits are conducted to assess laboratory practices and procedures, laboratory space and set up, laboratory information systems, quality management systems, collaboration with surveillance system and contribution to verification. Visits are conducted jointly between WHO and representative from an RRL or the GSL, or with representative from the RVC. Some visits have been conducted by RRL or GSL staff alone on behalf of WHO. RRLs have all been assessed jointly with the WHO Global Laboratory Coordinator because they are under the responsibility of WHO HQ. For 2019, based on 2017 performance for PT, all laboratories except one (72/73) were fully accredited for measles, with one laboratory provisionally accredited. All laboratories were fully accredited for rubella serology (73/73). Thirty-three of thirty-four laboratories were accredited for measles RT-PCR; 26 of 30 laboratories for measles sequencing; 29 of 32 for rubella RT-PCR, and; 17 of 19 for rubella sequencing.

In 2017 the workload for measles serology was 37,192 IgM tests (37% positive) and 26,322 rubella IgM tests (2% positive). A similar positivity rate is expected for 2018. The low proportion of rubella positives is due to some countries reporting results from pregnant women screening programmes. In 2017 the workload for RT-PCR for measles was 8,264 tests (31% positive) and 2,390 for rubella RT-PCR (1.7% positive). 2,533 measles strains were sequenced by NLs and RRLs, and 3,336 MeV sequences were reported to MeaNS. The higher number reported to MeaNS was due to reporting by sub-national laboratories in Italy. The number of rubella strains sequenced was 25, with 11 reported to RubeNS. To date in 2018, 7 RuV sequences have been reported to RubeNS.

Almost all MeV named strains reported to MeaNS since 2014 have belonged to B3 and D8 genotypes, with a few that belong to genotype H1. There are a number of none-named MeV strains, often because they are not present in sufficient number in the database. Seven Member States that reported cases have no sequence data available in MeaNS. Of the 39 countries that reported measles cases and also submitted measles sequences to MeaNS in 2017, only 16 complied with the WHO performance indicator for reporting

sequences (≥80% reported within 2 months of receipt of specimen). All countries that have generated sequences but not yet shared the data were encouraged to submit them as soon as possible.

In 2017 data presented to the RVC demonstrated that the majority of Member States (46 of 53, or 87%) met the 80% target for the rate of laboratory investigation of reported measles cases. Four Member States did not meet the target, and there were 3 countries with no available data on laboratory investigations. The majority of Member States (34 of 53, or 64%) use IgM serology as the first-line testing strategy. Fifteen countries (28%) use a combination of serology and molecular testing, and 4 (7.5%) exclusively use molecular testing for laboratory investigation. The latter strategy is not recommended, and participants were welcomed to discuss this issue.

In 2017, 29 of 44 Member States (66%) that reported measles cases met the 80% target for genotyping and characterization of chains of transmission. One challenge to meeting the target is that some laboratories receive specimens without epidemiological data on the possible chains of transmission, and if the laboratories cannot sequence all specimens received, isolates for sequencing are randomly selected. This can result in several chains of transmission having no representative strains sequenced. Participants were requested to consider potential strategies that the laboratories could adopt in such situations. Genotyping data alone is not now sufficient to determine and understand pathways of MeV transmission, and it is strongly recommended that isolates are sequenced and named strains used.

Though the latest round of verification shows that 72% (38 of 53) of Member States met the 80% target for laboratory investigation of rubella in 2017 and are doing well, the majority of rubella cases reported in the region are clinically compatible and are reported by a small number of countries. Only 17% of reported rubella cases were laboratory confirmed. Correct specimens are often not collected. For this reason the data on rubella sequences in RubeNS is very sparse.

2018 has been a difficult year for WHO to organize the procurement of ELISA diagnostic kits for measles IgM and rubella IgM for the countries that are supported. DiaSorin, which acquired Siemens may cease production of the kits in 2019 and a decision has been made to move to Euroimmun kits. The manufacturer has been responsive and conducted a validation study for use of the kits with dried blood spots (DBS) for measles and rubella IgM. The kit was placed into the WHO centralized GSM procurement catalog and shipped to the supported countries, but challenges were faced with customs clearance of shipments, and with recall and replacement of a batch of defective kits. An additional challenge is that the kit is not validated to be used for dried serum spots (DSS) which is an important issue for a number of countries, particularly for retesting by RRLs. A large-scale comparison study is being conducted at global level with the expectation of validating a number of kits from different manufacturers. This will allow more options for NLs and also for WHO to supply the countries.

Discussion

- The evaluation of the Euroimmun kits on DBS was conducted by the manufacturer on PerkinElmer paper but it appears there is little difference between Whatman and PerkinElmer paper. However, an evaluation study with Whatman paper will be necessary. Results of the evaluation will be shared with the MR LabNet.

- Information on the strategy being used at the Moscow RRL for selecting specimens to be sequenced when epidemiological data was not available was shared. When a laboratory receives many specimens from the same location, the specimens are stored and, after potential chains of transmission are identified by epidemiologists, the laboratory recovers the specimens and sequences those from chains with isolates not yet sequenced.
- In outbreaks there is no need to do IgM testing on all cases. To avoid wasting resources and causing issues with laboratory capacity, it is recommended to request that data be provided on epi-linked cases. When calculating the rate of laboratory investigation epi-linked cases are excluded from the denominator.
- The 2nd version of MRLDMS will include an option to do bulk upload, this option and is not ready to be released yet. In a meantime, especially during outbreaks, it is sufficient to report monthly aggregated results to CISID. In the future, when MRLDMS is fully operational, reporting to CISID will discontinue. EURO continues to use several channels of information and laboratories are requested to exchange laboratory information with their epi colleagues at the national level before reporting data to WHO. The Regional Office is working together with ECDC to have a single point of entry for laboratory and epi data reporting, but it is challenging to accommodate the two systems with different legal requirements for reporting in some countries.

4.4. Update from the Regional Verification Commission

Dr Irja Davidkin, RVC member, provided an update from the RVC. The 7th RVC meeting took place in Paris on 13-15 June 2018. This was the first time the RVC received ASU reports from all 53 Member States, although some were received after the deadline. In general, the quality of reports has continued to improve, but information required to assess the sensitivity of surveillance systems was inadequate or lacking from several reports. Changes were made to the ASU laboratory sections for 2017 and all laboratory information is now grouped in the same place. Molecular epidemiology of chains of transmission and of sporadic cases is now provided in the same table.

The RVC concluded that, by the end of 2017, 43 Member States provided evidence to demonstrate that endemic transmission of measles was interrupted. Of these, 37 have eliminated endemic transmission for at least 36 months. Endemic rubella transmission was interrupted in 41 Member States, of which 37 have eliminated endemic rubella for at least 36 months. Thirty-five Member States provided evidence for the elimination of both measles and rubella.

The RVC again noted that despite improvement the extent and quality of rubella and CRS surveillance remains suboptimal in many countries. While efforts to provide retrospective analysis of potential rubella cases to provide supplementary evidence for the absence of detected disease are applauded, the RVC reminded Member States that these studies are not currently acceptable as alternatives to mandatory reporting of rubella cases and nation-wide rubella and CRS surveillance.

The ability to distinguish between remaining endemic transmission, imported-related sporadic cases and import-related outbreaks is now crucial to the verification process, and monitoring chains of virus transmission through genomic sequence analysis is essential. All Member States were reminded of the requirement for all measles and rubella isolates from sporadic cases, and representative isolates from

outbreaks, to be submitted for genomic sequencing. In reviewing the 2017 reports the RVC relied on the measles isolate genotyping data to determine if the evidence supported a conclusion that endemic transmission had been interrupted or, in the case of countries previously regarded as having interrupted transmission, had not re-established endemic transmission. Most Member States now report their measles virus genomic sequence data to the measles nucleotide surveillance database MeaNs, but the amount of sequence data reported to the rubella nucleotide surveillance database RubeNs remains low.

4.5. MeaNS and RubeNS update

Dr David Williams, PHE, London, UK provided an overview of MeaNS and RubeNS. As of November 2018, there have been approximately 43,000 sample records submitted to MeaNS, almost double the number submitted up to 2016. RubeNS contains only 2,631 sequences, but the number of rubella sequences is slowly increasing. Of the 24 measles genotypes originally recognized, only 5 have been since 2015 (B3, D4, D8, D9, H1). B3 and D8 are the predominant genotypes in the European Region, with a small number of imported H1 isolates, mainly from China. The most recent named strains of B3 and D8 cluster in small groups within the genotypes, so that even within genotypes only a limited number of predominant named strains can be detected. This is evidence that the programme is beginning to breaking chains of transmission and measles elimination is in process.

There are currently no named strains within RubeNS because so few sequences are available, which has been an ongoing problem. There were only 7 rubella sequences submitted from the European Region in 2018, and NLs have been requested to send specimens to the supervising RRL or to GSL, if they cannot conduct sequencing themselves.

MeaNS and RubeNS have been invaluable tools for monitoring virus transmission, documenting importations and monitoring loss of endemic viruses within countries and at regional and global level. Now MeaNS has a large repository of data, but unfortunately the data is very heavily sample biased. In the past it was dominated by data from the UK because of the sequencing strategy in place there. Now data from many European countries are well-represented, but there continue to be large parts of world for which there is very little data. Because of this the data from MeaNS cannot be used to accurately interpret the global situation with regard to transmission. While RubeNS can be useful for verification of a country elimination status, the strong sampling bias makes it unsuitable for broader interpretation.

For the ASU the NL needs to provide genotype and sequence ID information in order to identify strains in circulation across the region as a whole. MeaNS and RubeNS, however, are running on outdated software, the site sometimes runs very slowly and the databases are very labour intensive to maintain. There is an additional problem in that errors generated by GenBank change the WHO names. In MeaNS and Rubens samples are saved based on the WHO name, but when uploading sequences from GenBank into MeaNS and RubeNS a significant amount of checking needs to be done. For these reasons among others, MeaNS and RubeNS are being updated.

The core layout and usage will be unchanged, e.g., to submit a record: ‘Data’ → ‘New Record’ → fill form. Page loading and database searching will be faster, with bigger text etc. There will be some additional tools that are being developed (e.g. an increased ability to conduct sequence searches and phylogenetic

reconstruction, and to generate world map summaries in real-time). It is anticipated that a draft version will be built by the end of 2018. From that point, data from the old MeaNS and RubeNS that will continue to be fully functional for the following six months, but then transfer to the new MeaNS-2 and RubeNS-2 will take place. Part of the process will be a checking of old data for errors (removing duplicates, correcting WHO names). The data submitters will be contacted if needed, e.g. to update/correct the information; and especially in case of duplicate sample uploaded from GenBank to clarify which is the correct one. Ideally, MeasNS-2 and RubeNS-2 will be launched at the next Global LabNet meeting in summer 2019.

Discussion

- The sequence ID and the named strain should be visible if the user uses 'NL listing' and then downloads.
- There are no tools in MeaNS and RubeNS to download sequences. This decision was made early in the development of MeaNS and RubeNS to prevent users from downloading sequences. These databases are tools for WHO that contain sensitive country-specific information that is not in the public domain. Any user wishing to use data in their own publications needs first to contact the data submitter requesting and obtaining permission to use the data.
- There remains some debate on how to manage the WHO names in the new databases, with constraints placed on the naming convention. There are currently significant differences in the naming conventions used by different countries with regard to city, county, state or province. In the revised versions of MeaNS and RubeNS capacity will exist to correct a WHO name if the submitter has made a mistake.
- There are some WG sequences in MeaNS and in RubeNS, and users can currently import WG sequences. Increasingly labs are using MF-NCR and WGS, and this functionality will be part of the new versions.
- At the moment a distinct sequence ID works well for N-450, but how we interpret and use MF-NCR and WGS is less clear. It is also unclear if use of a distinct sequence ID will be beneficial.

4.6. Public Health England – Global Specialized Laboratory update

Dr Kevin Brown, Public Health England, London, UK, provided an update on the Global Specialized Laboratory. Separate health authorities in the UK report to the WHO through Public Health England (PHE), which is now part of the Department of Health, and is responsible for public health, health inequalities and vaccine procurement/delivery. Major restructuring and cost saving actions are on-going in PHE and a new Clinical Services Unit has been created. A National Infection Service has been formed, the laboratories are no longer independent and have become a 'commissioned service' instituting more close collaboration between laboratories and epidemiologists.

OF testing is the basis for surveillance for measles, mumps and rubella in the UK. Most OF samples tested for measles are negative, but negative results (discarded cases) cannot be reported according to national regulations. At the start of 2018, isolates belonging to genotype B3, particularly the named strain MVs/Dublin.IRL/8.16/, were identified in importations from Romania, but towards the end of the year there was a switch to isolates belonging to genotype D8, both named and unnamed strains, mainly associated with importations from France. It is currently very difficult to prove that there has not been

continuous circulation of the same strains from mid-2017 to mid-2018. Detection of vaccine genotype A isolates occur throughout the year, indicating that high quality surveillance is in place.

Currently, N-450 is the primarily region used for MeV genotyping. However, this is not the most variable part of the genome. The majority of strains collected during the D8 outbreak in 2012/13 have N-450 sequences identical to the WHO named strains MVs/Taunton.GBR/27.12/ and MVs/Swansea.GBR/4.13/. The single nucleotide difference found between these two is most likely a result of stochastic viral mutation during endemic transmission rather than of multiple importation events. Analysis of the H gene does not provide significant further separation of the analyzed strains and the phylogenetic tree obtained from analysis of the entire N gene appears to produce better resolution compared to that obtained from analysis of the H gene.

The MF-NCR region, located between the M and F genes, is more variable than N-450 and offers the potential to distinguish endemic from imported MeV. WGS, or more specifically WGS excluding the 3' and 5' termini (WGS-t) also offers good potential for higher resolution analysis of phylogeny. BEAST analysis was used to obtain an estimate of the substitution rate observed at the MF-NCR and WGS-t, with substitution rate highest for MF-NCR. This rate was approximately 3-fold higher than that for N-450, suggesting that it may be useful to distinguish between identical N-450 or isolates with small changes in N-450. Work is ongoing to improve the PCR for MF-NCR. The GSL is receiving more WGS-t and MF-NCR sequences that are now being published.

Computer modeling of the expected number of substitutions per unit time can be used by laboratories to determine, within 95% confidence limits, if sequences from different isolates are linked or not linked, i.e. if they are more likely to be in the same or separate chains of transmission. This may be a very useful way to interpret data and if it can be easily modeled, it could be added into MeaNS as a tool. It should be noted that a range of expected substitutions can normally be observed in different regions of genome and that analysis of linkage is probability-based, not an absolute measure. Typically, no changes would be observed in the N-450 region within a year, with fewer than 5% of samples are expected to have more than 3 substitutions. For the MF-NCR region, 2 samples in the same transmission chain would be expected to have 7 differences between them, and up to 95% of samples would have at most 13 substitutions.

Whilst widening the sequencing window allows better characterization of MeV outbreaks, N-450 remains the best approach in low-resource and endemic transmission settings. The most detail can be obtained from WGS, but this is expensive and time-consuming. MF-NCR sequencing may complement N-450 in well-resourced countries approaching elimination, particularly to identify importations.

Several years ago, PHE identified a problem with the MicroImmune IgM assay, which worked well on serum samples but was problematic with OF samples. It has become clear that FCS should be used as OF diluent to avoid false positive results, that if samples have insufficient IgG or have been collected improperly, both false negative and false positive results are possible. There is a need to modify the instructions on minimum concentration of total IgG so that test over cut-off ration is 3, rather than 1 as currently indicated by the manufacturer. Clin-tech has modified the English version of instructions, but the French version remains uncorrected. There is also a need to replace the test over negative ration in the instructions with test over cut-off, together with interpretation of the results. Clin-tech was producing the MicroImmune assay but it has been sold to Calibre Scientific, and the future availability of the assay is unclear.

From the 1st of April 2016 antenatal screening for all pregnant women in the UK was stopped. The current recommendation is for non-vaccinated women to be vaccinated post-partum. There has been a slight increase in the number of IgM samples from pregnant women aged 15 to 50 since cessation of routine screening, but does not seem to present a problem. Only 1 case of rubella in the UK was reported in 2018. This occurred in a pregnant woman infected in Algeria (genotype 2B). A second case (an importation from Romania) remains under investigation. Participants were alerted to be aware of a number of publications now describing granulomatous disease with rubella infection in children with primary immunodeficiency. Most of the cases in the literature are children with ataxia telangiectasia, who developed granulomatous lesion triggered by rubella vaccine. The rubella sequence can be generated from granuloma tissue, and in the US rubella virus has been cultured from the lesions. There has been a single case in the UK with wild-type RuV identified from granuloma material.

4.7. Regional Reference Laboratory – Luxembourg update

Judith Hübschen, RRL, Luxembourg, provided an update on laboratory activities. In 2018 the RRL received 566 measles samples, the most ever received in a single year, but the number of samples received for rubella testing decreased to 342. Samples were received from a total of 23 NLs. Measles confirmatory results were generally good, but 6 laboratories sent <10 specimens and major discrepancies were found in the results from 3 laboratories. An overall >95% concordance was reached. Ten laboratories sent <10 specimens for rubella testing and only one major discrepancy was observed.

The RRL received 401 DSS samples for retesting compared to 367 liquid serum samples. In addition, the laboratory received 65 OF samples and 75 DBS samples. Testing DSS has become a very important part of confirmatory testing for the Luxemburg RRL and a switch to the use of Euroimmun kits requires establishment of a new protocol for DSS testing. The RRL has been working on this protocol and is investigating the methodology on the basis of sample stability and intra-assay reproducibility. The RRL is continuing to work on the new protocol and will report results at a future meeting.

In 2018, approximately 390 samples were received from 6 different NLs for molecular detection and sequencing, with an additional 20 samples from Luxembourg. Samples from approximately 150 patients from Bosnia and Herzegovina and Serbia have investigated by the MF-NCR single fragment PCR and 90 sequences were obtained and analyzed to date.

An historical collection of 350 rubella IgM positive sera, collected up to 9 years previously in the Central African Republic and stored at -80°C, were sequenced at the RRL. Thirty-six full length 739 nucleotide sequences were obtained. In 2017 the RRL also received 21 sera from one lost shipment, received after 1 month in transit, and managed to obtain 7 sequences. These results demonstrate the value of sequencing as a confirmatory test and if laboratories are not able to sequence from available serum samples, they should forward these samples to the RRL or GSL for testing.

A MR seroprevalence study, using data already available, has been conducted by the RRL. Measles and rubella IgG test results obtained by diagnostic laboratories in Luxembourg over a 10-year period were requested and 6 laboratories agreed to participate, sending a total of 94,081 results. After exclusion of duplicates, multiple test results and data lacking information on age, gender, place of residence and test date, approximately 71,800 entries (2,800 for measles and 69,000 for rubella) remained for analysis. The

large number of results for rubella is due to the prenatal screening programme conducted in Luxemburg until 2015. It is expected that many other countries in the Region have similar available laboratory data that can be analyzed and used to provide evidence in the ASU.

Discussion

- The Luxemburg RRL has been evaluating use of DSS with Euroimmun; the manufacturer has already conducted evaluation with DBS on PerkinElmer paper and no issues of specimen stability have been reported. There is a need to evaluate use of DBS on Whatman paper (which is commonly used in the LabNet) using Euroimmun. Stability of antibodies in DSS is an issue, but is not an issue in DBS because of the higher protein levels. It may be possible to add a stabilizing agent to DSS to increase stability and decrease the rate of specimen deterioration.

4.8. Regional Reference Laboratory – Berlin update

In place of the usual Berlin RRL update Dr Sabine Santibanez, RKI, Berlin, Germany, presented details of a project on MF-NCR sequencing at the Berlin RRL. This was the first attempt of the laboratory to use this method to distinguish between separate transmission chains among measles virus variants with identical N-450 sequences. In 2015 Germany hosted approximately 2 million international migrants and refugees. On arrival migrants stayed in centers for asylum seekers before moving out to fixed places of residence across Germany. A D8 Rostov on Don variant was first detected in Germany at the end of 2013 and from there most likely exported to Bosnia and Herzegovina, where a large measles outbreak with >8,000 cases occurred from February 2014 to the end of 2015. There were separate, repeat importations, mainly from Bosnia and Herzegovina, into Germany starting in the summer of 2014. Several outbreaks of D8 Rostov on Don were detected, including one in Berlin-Brandenburg with approximately 2,000 cases. It was estimated that approximately 2,400 cases were associated with D8 Rostov on Don. While D8 Rostov on Don was the major variant detected, viruses distinct from but closely related to it were also identified. Many variants that initiated transmission chains had circulation periods of 3 to 5 months, but several transmission chains of D8 Rostov on Don appeared to have circulation periods of 13 to 14 months in Germany. Questions were raised over the possibility of distinguishing between transmission chains involving apparently identical viruses and of linking a transmission chain to a particular importation event. MF-NCR sequencing was investigated as a method for providing potential answers to these questions.

The project was conducted within the activities of WHO Global Measles/Rubella LabNet working group N.E.W. Protocol using a primer modified at RKI. vRNA from 239 D8 Rostov on Don-associated cases, from samples collected in Germany between summer 2014 and summer 2015, were sequenced. From these 55 separate MF-NCR variants were identified. The distribution of sequence variants over time suggested the presence of 3 major variants with long-lasting circulation. Variant 1 was detected over a 7-month period and linked to 69 cases. Variant 2 was also detected over a 7-month period and linked to 43 cases; and variant 3 was detected over 8 months and linked to 81 cases. A number of other variants were detected only for short time periods. Since there were multiple importations of D8 Rostov on Don into Germany from Bosnia and Herzegovina and Serbia, it was decided to compare sequence data from these two countries with data generated in Germany. Phylogenetic analysis of the three variants suggested significant genetic difference between them and also differences in geographic distribution. Variant 1 and its descendants were found in Germany and Serbia, but not in Bosnia and Herzegovina; variant 2 was detected

in all three countries, and; variant 3 was only detected in Germany. Sequencing of samples from Bosnia and Herzegovina and Serbia was conducted in the RRL in Luxemburg. Descendants of each of the major variants were detected, but differences between descendants and the major variant is very low (1nt difference). Unambiguous identification of transnational transmission chains is not yet possible since the data was not collected systematically and additional representative data from MeV-exporting and -importing countries is required. In particular, systematic investigation of the initial phase of an outbreak is necessary. In addition, interpretation of the degree of change in the MF-NCR region is difficult. The estimation of likelihood of nt substitution along a transmission chain through epidemiological analysis of confirmed chains has been suggested as a way of interpreting this.

Discussion

- The primers used for MF-NCR in the GSL London cover the same MF-NCR region but are slightly different to primers used by the Canadian Public Health Agency. It is possible that laboratories will want to use slightly different primer sequences for MF-NCR if they are doing D4, D8 and B3. It is useful to first sequence N-450 and then optimize what to sequence rather than using primer pairs that cover all different genotypes. RKI Berlin uses the same protocol as the Canadian Public Health Agency, but has modified one primer.

4.9. Regional Reference Laboratory - Moscow update

Dr Tamara Mamaeva, provided an update on the RRL, Moscow. The Moscow RRL serves 9 NLs in the Commonwealth of Independent States (CIS) and 11 SNLs, 10 of which are in Russia and 1 in Kyrgyzstan. ELISA is the common method of laboratory testing, using ELISA kit suppliers in accordance with WHO laboratory accreditation criteria. Until 2014 the laboratories were using their own in-house controls, but in 2014 to 2015, panels of laboratory controls for non-quantitative investigation of measles IgM and IgG and rubella IgM and IgG were commercially prepared by Vector Best (RF) for use in CIS and RF laboratories. The controls consist of lyophilized human sera inactivated by heating. Reconstituted aliquoted samples are stored at -20°C and are stable for at least 3 years. In the CIS LabNet the variability rate with different assays has been below 20%. and interlaboratory reproducibility has been high.

The laboratories entered their serology PT results online for the first time in 2015. 52% of CIS labs made submission errors and difficulties were observed with kit validation criteria, cut-off values, in-house controls and kit expiry dates. These errors influenced the overall score though all samples were correctly identified. There were fewer errors in 2016/17. 10 of the SNLs in RF have received and tested the 2018 PT panel, with one laboratory reporting a measles IgM discordant result. All 10 SNLs entered kit data and validation information, as well as in-house controls, without errors. There was 100% concordance between the NLs and RRL, and between all Russian SNLs and the RRL in the last confirmatory testing round both for measles and for rubella. Concerns have been raised, however, over the variety of commercial kits used in the CIS LabNet, and the large number of measles positive samples sent for retesting in 2018. In total in 2018, 926 measles samples were sent to the RRL for retesting, of which 428 (46.7%) were positive for measles. Of the 818 rubella samples sent, only 22 were positive (2.7%). 4 NLs sent DSS for retesting, amounting to 23.8% of all samples for retesting.

In 2018 the NLs were offered the Euroimmun kit, and the RRL has prepared a protocol for automatic calculation and interpretation of test results in accordance with the instructions. Instructions for both

qualitative calculations using a calibrator OD as cut off, and a semi-quantitative method which calculates ratio (sample OD/calibrator OD) have been provided. It was noted that the Euroimmun kit, similar to the Siemens kit, is an indirect assay and the sample buffer contains IgG/RF absorbent. Incomplete removal of IgG from serum may result in errors. When testing serum samples collected within the first 3 days of onset of rash, 20-30% of cases may produce false-negative results. Some NLs of CIS countries receive a large proportion of samples collected <4 days of rash onset, so it may be necessary to use a more sensitive immunoassay. It has been noted that some samples give discordant results when tested using different kits. These samples were often from previously vaccinated individuals, with the presence of measles-specific IgG causing false negative IgM results with some kits.

In the first 8 months of 2018 referral samples from approximately 6,000 suspected cases (half of which were suspected measles) were tested in RF. Of these 38.5% were positive for measles IgM. The RRL will provide all supported laboratories with an Excel program for calculating and interpreting testing results with the Euroimmun kit. Commercial in-house controls prepared by Vector Best have been validated now for use with the Euroimmun kit and all CIS NLs will receive recommendations from the RRL on how to use them. The variety of kits used for measles and rubella diagnosis is increasing in the CIS network, not only for IgM, but also for quantitative and qualitative detection of IgG. There is a need for a new protocol for use of DSS with the Euroimmun kit. The RRL has been working on a protocol and will provide recommendations in due course.

Dr Sergey Shulga, RRL, Moscow, provided an update on genetic monitoring of circulation of MeV in the RF and CIS. In 2015, due to a significant reduction in circulation of endemic D8 variants followed by a similar low incidence in 2016, it was anticipated that verification of elimination in the RF could be achieved by 2018. Unfortunately, from the end of 2016 an increase in measles cases is observed. In 2017, 721 measles cases (4.9/million population) were reported, with the majority of cases 612/712 (85%) reported in 4 territories. Epidemiological investigation indicated that apart from a large number of local cases, there were 22 imported cases. It is not possible to characterize all imported cases based only on epidemiological data because the RRL frequently identifies genetic variants that have not previously been detected in the RF. A total of 15 genetic variants were detected, with the majority (10) being identified for the first time in RF. At the start of 2017 there was moderate circulation of D8 variant Frankfurt, with increasing measles incidence in the second half of the year associated with importation of B3 variant Dublin. Circulation of this strain continued into 2018. Until August 2018, 2,177 measles cases were reported (1.5/million population), with the majority of cases (72%) reported in 6 territories. Two measles genotypes were identified, B3 and D8, and epidemiological investigation has suggested fewer imported cases compared to 2017. A total of 22 genetic variants have been identified, some of which appear to be new derivatives of the main D8 Frankfurt strain, which has continued to circulate in RF for more than 2 years. At the same time there were sporadic cases and chains of transmission linked to genetic variants not seen before in the RF and assumed to be associated with imported cases. The D8 Frankfurt variant was one of the dominant variants from 2012 to 2014, but circulation was assumed to have been stopped from mid-2015 as it was not reported in any of EUR countries for 3 to 5 months. The strain reappeared in 2016/17, however, and has since been frequently detected in the RF in connection with the Caucasus republics in the southern part of the country. Analysis of the phylogenetic tree of D8 Frankfurt and its descendants suggest a large number of sporadic cases and chains of transmission were internally-imported from these southern republics. In 2017 it was possible to connect approximately 200 cases with a Frankfurt variant. In 2018 there have been more cases, with 11

variants of this high heterogenicity cluster being identified. Other D8 variants identified in 2018 were D8 MVs/Gir Somnath.IND/42.16/, MVs/Samut Sakhon.THA/49.16/, and MVs/Cambridge.GBR/5.16/. All of these were repeatedly imported from different countries, most circulating only for a few weeks, but D8 Gir Somnath has maintained circulation for 25 to 26 weeks.

With regard to genotype B3, the dominant named strain identified is MVs/Dublin.IRL/8.16/ which has been detected for more than 12 months, mainly in the Moscow region. Almost all of these cases were considered to be local cases, although repeat importations cannot be excluded.

In 2018 samples for molecular investigation were received from Armenia, Uzbekistan, Republic of Moldova and Kyrgyzstan. Before 2016, the outbreaks in CIS were characterized by circulation of a single imported genotype variant with little variability or heterogenicity observed. In 2018, however, almost all countries had more than one genetic variant identified, suggesting that even when measles incidence is low, and epidemiological data shows few localized clusters, it is advisable to conduct more detailed investigation as co-circulation of several genetic variants is a possibility.

Discussion

- A question was raised over interpretation of laboratory results of reinfection (secondary vaccine failure) cases to the epidemiologists and clinicians. Every territory in RF has a committee comprised of epidemiologists, clinicians and representatives from the laboratory. Based on all available data the committee makes a final case classification. In Russia, at least monthly explanatory meetings are held with epidemiologists and clinicians to interpret the laboratory results of difficult cases. In the past it took some time for the RRL to clarify false positive and false negative measles cases with the epidemiologists. Regular explanatory meetings and development of algorithms together with the epidemiologists is the only solution.
- Concerns were raised over choice of method for calculating results using the Euroimmun IgM kit. Both the RRL Luxemburg and RRL Berlin use a semi-quantitative method for results calculation (ratio interpretation: <0.8 is negative, ≥0.8 < 1.1 equivocal and ≥1.1 positive).

4.10. Introduction to the 4th round of mEQA for measles and rubella

Dr Bettina Bankamp, CDC Atlanta (via WebEx), provided an introduction to the 4th round of mEQA for measles and rubella. CDC have been administrating the WHO mEQA programme for other WHO regions for 7 years. It was decided that EUR would switch to WHO/CDC to improve harmonization of results and have a single globally distributed panel. CDC recommend using QIAamp Viral RNA Mini kit for RNA extraction, but there is an option to use the INSTAND elution method. Participating laboratories need to send completed reports and supporting data to CDC by email within six weeks of panel receipt. Chromatograms and cropped sequence text files need to be submitted to the MeaNS or RubeNS mEQA sites. The measles and rubella teams at CDC evaluate the report and prepare a feedback report with the final score. Scores are: Pass/Retest/Fail. If the result is 'Retest', each panel contains two sets of samples (the backup samples) to allow retesting, usually it is not necessary to repeat everything, but only the part that did not work well. If the result is 'Fail', the Global and Regional Coordinators will discuss options for corrective actions with the laboratory, including providing additional training. A practice panel can be sent after the corrective action is complete.

Requirements for obtaining a 'pass' score for detection include correct detection of measles or rubella RNA (or negative result) in all of the samples and adequate positive and negative controls in the PCR assays. Requirements for obtaining a 'pass' score for sequencing include correct identification of the measles or rubella genotypes in each positive sample; complete sequence for the entire sequencing windows for measles (N-450) and rubella (739 nucleotides) and sequences submitted to MeaNS and RubeNS; no nucleotide errors when compared to the reference sequences. Retest are offered if the problem can be resolved by repeating a test, e.g. re-analysis of sequencing data. Failure calls for training or a change in workflow, e.g. invalid real-time assay. All laboratories may receive comments in the comments section that do not affect the score, e.g. issues with WHO names. The laboratories should read the comments carefully and take note of the issues.

Discussion

- The requirement for laboratories that only perform PCR in shipping the PCR product to their RRL is to ensure that the PCR product can be sequenced. The RRL should be able to get a sequence if the PCR product is of sufficient quality and quantity. However, not all RRLs in the region have the time or personnel capacity to do sequencing for these laboratories solely for mEQA.
- In other regions the RRLs send chromatograms back to the NLs for sequence analysis if they have sequence capacity. In EUR all RRLs analyze chromatograms themselves, there is little expertise for sequence analysis in most of the laboratories that forward samples for sequencing to the RRL. WHO/CDC and EURO will further discuss the issue of shipping PCR products and forwarding chromatograms to the RRLs. Procedures may need modification for EUR.

4.11. Presentation of WHO Laboratory Manual, 2018

Dr Mick Mulders WHO HQ, Geneva, presented the main features of the new Manual for the Laboratory-based Surveillance of Measles, Rubella, and Congenital Rubella Syndrome 3rd edition[2]. The manual outlines the activities of the laboratory network in support of immunization and surveillance programmes and goals, including the disease elimination verification process, and describes the accreditation and quality assurance programmes. It provides guidelines and best practices for collecting suitable clinical specimens and the laboratory confirmation of measles and rubella infection in different settings, together with guidelines, tools, forms, and protocols for diagnosis of measles and rubella infection and molecular characterization of circulating viruses. Annexes include protocols that have been developed and made available by the laboratory network. Participants were invited to share any protocols that they feel are important for the network for posting in this manual website.

4.12. Progress on Measles and Rubella Kit Comparison and Evaluation

Dr Mick Mulders WHO HQ, Geneva, described progress made on comparison and evaluation of different measles and rubella immunoassay kits. There is an urgent need to replace the Siemens kit used in the GMRLN as there is no certainty that this kit will be available after 2019. Serology remains the gold standard for measles and rubella diagnostics, and there is a need to have several kits evaluated and available in the WHO GSM catalogue. In EUR many of the laboratories already use Euroimmun kits in testing for other infections, so introducing these kits for measles and rubella should be relatively easy. In other regions,

[2] https://www.who.int/immunization/monitoring_surveillance/burden/laboratory/manual/en/

particularly in Asia, the Virion Serion kit is used and experience has shown it to have good performance. The reliability of the Microimmune kit is unclear since production has been acquired by another company. Some countries have had good experience with the NovaLisa kit, which is an indirect assay. Alternative kits have been listed in the WHO catalogue, such as the Virion Serion kit which was added 15 years ago, but the general performance of this assay is unclear.

WHO will conduct a study of commercially available kits to evaluate the accuracy of measles and rubella IgM and IgG assays compared to established performance criteria. Kit evaluation includes a review of validation studies undertaken by the manufacturer who will be required to provide data to WHO confirming the manufacturers' ability to maintain continuity of global supply. Manufacturers should have the capacity to cover at least 60 to 70% of the current global needs of the network. Testing of kits will be conducted at the National Microbiology Laboratory, Public Health Agency of Canada. To date products from 5 different producers are expecting to be compared: for measles IgM - Euroimmun (recombinant vs. whole antigen), Microimmune, Virion Serion, NovaTec, and Cadilla (this is manufacturer in India); for rubella IgM - Euroimmun, Microimmune, Virion Serion, and NovaTec. Evaluation of assays for IgG antibodies will be conducted at a later data. A draft evaluation protocol has been written and available. Formal *Expressions of Interest* have also been prepared and are ready to be shared with manufacturers.

Some small comparison studies have already been conducted, including one at the RRL in Oman (Siemens) that was investigating confirmatory testing of samples coming from the NL of UAE (Virion Serion). Discordance was found to be 39/43 (90.6%), but from 4 discordant results only one was a major discordance. Concordance of 90-95% is considered normal. Experience of the Egypt laboratory, which switched to Virion Serion in 2018, have shown several invalid runs (8 out of the first 12 test-runs). Furthermore, even in valid runs, a high proportion of equivocal results and some false positives were found. The FioCRUZ laboratory, Brazil, conducted a comparison between Euroimmun and Siemens measles IgM kits using 170 sera collected during a measles outbreak in 2018. All 170 sera were tested using the Siemens kit, and 141 of these were also tested using real time PCR. The concordance of the results was good, with only 2 significant and a small number of minor discordances. The ISP laboratory in Chile conducted a small comparison study between the Siemens and Virion Serion kits, with all 40 results concordant for measles IgM and 2 out of 40 discordant results for rubella IgM. The National Institute of Health, Thailand, conducted a comparison study of Siemens versus the Euroimunn (wells coated with recombinant MeV NP) and Siemens versus Euroimmun kit (wells coated with whole antigen) using 200 serum samples. In both cases the concordance was 94%, with lower sensitivity for the Euroimmun kit coated with recombinant NP (92.63%; with whole antigen - 94.74%).

Dr Elina Horefti, NL Greece, presented details of a small comparison study between the Siemens and Euroimmun measles IgM assays. 12 samples were tested with no significant discrepancies, but 3 samples gave results in the grey zone with Siemens, turning weak positive with Euroimmun, and 1 weak positive sample with Siemens in the grey zone with Euroimmun. All 4 discrepant cases were RT-PCR positive.

Dr Victoria Indenbaum, Israel, presented details of a comparison of the Siemens, Virion-Serion, Biorad Platelia, Euroimmun NP and Euroimmun (whole antigen) measles IgM assays conducted in 2018. 11 samples were true positive (positive in qPCR or with clinical signs from an outbreak), 4 samples were true negative. There were some discordant results where samples positive in Siemens, Virion-Serion, Biorad Platelia, were negative in Euroimmun, suggesting lower sensitivity. There were 2 true positive samples

where the Siemens kit showed a negative result (near grey zone) and the Virion-Serion kit detect antibodies (one of the cases as equivocal). These 2 samples were collected 1 to 2 days post rash. A similar comparison study was conducted in 2015 between Siemens and Euroimmun kits and also suggested a lower sensitivity for the Euroimmun kit.

Session 5 – Country presentations

Chair: Judith Hübschen (RRL, Luxemburg)

5.1. Studies into the mechanism of measles-associated immune suppression during a measles outbreak in the Netherlands

Dr Rik L. de Swart, Erasmus MC, Netherlands, described studies on measles-associated immune suppression in the Netherlands. Measles is associated with a transient immune suppression, resulting in increased susceptibility to opportunistic infections, such as pneumonia, gastrointestinal disease and otitis media, that are largely responsible for measles-associated morbidity and mortality. Previous studies in non-human primates suggested that MeV predominantly targets CD150+ immune cells and that the primary site of virus replication is primary and secondary lymphoid tissues. Histopathological analyses have shown that as result of infection, lymphoid tissues become disorganized and show B-cell follicle depletion around the peak time of virus replication. An *immune amnesia* model has been developed to explain the mechanism of measles immune suppression. Measles is associated with lymphopenia that lasts for approximately 1 week, with immunosuppression of longer duration. Previous epidemiological studies have shown that the incidence of measles is tightly associated with overall childhood infectious disease-mediated mortality, and the period of increased risk of childhood mortality seems to extend over a period of >2 years. The conclusion from this study was that a substantial number of deaths that are not registered as measles may, in fact, be directly related to a previous measles episode. A weak point of the study was that it was population-based. A new study has been conducted in the THIN (The Health Improvement Network), a general practice registry in the UK, using a case-based assessment of the impact of measles[3]. Measles cases were selected from a database and every case was coupled to a matched control from the same GP practice center. The study found that GP consultations peak immediately after a measles outbreak, but are also more frequent over the following 2 to 3 years for measles cases than in the control group. The same is true for the number of registered infections in children that contracted measles. It was concluded that measles infection has a prolonged impact on host resistance to non-measles infectious diseases.

Measles induces immune suppression, but also induces immune activation that leads to a very good immune response to measles itself. In 2013 a large measles outbreak, with at least 2,600 cases and possibly as many as >30,000 cases, occurred in the Netherlands. The outbreak occurred predominantly in the Dutch Orthodox Protestant community and provided a unique opportunity to study the pathogenesis of measles immune suppression in unvaccinated children. Children aged 4 to 17 years were studied. The group was divided into 2 cohorts: A - acute measles patients studied to assess the tropism of MeV; B – children without acute infection studied to assess the frequencies of lymphocytes, with bloods collected before and after natural infection. All acute measles patients (cohort A - 23 laboratory-confirmed cases) had classic

[3] https://bmjopen.bmj.com/content/8/11/e021465

lymphopenia with reduced T-cells and B-cells. Naïve $CD4^+$ T-cells included very few MeV-infected cells whereas a large proportion of memory $CD4^+$ T-cells were infected. The same was seen in $CD8^+$ T-cells, but infection levels were lower. Both naïve and memory B-cells became infected with MeV. In cohort B (42 paired serum samples collected before and after measles infection) the study found reduced frequencies of circulating memory B cells and increased frequencies of regulatory T cells and transitional B cells. The study concluded that measles viremia is mediated by MeV-infected memory T-cells and both naïve and memory B-cells, and that measles has a lasting impact on the distribution of circulating lymphocyte subsets. These data support the immune amnesia model as a mechanism of measles immune suppression.

Discussion

- The issue of the mechanism of vaccine MeV effects on the host immune system was discussed. Studies have shown that vaccine MeV is not commonly associated with viremia, and if it is observed, the level is several log values lower than in natural infection. It is assumed that virus infection of lymphocytes does not occur, but vaccine virus predominantly replicates in the myeloid cells and does not spread to lymphoid tissues (no lymphoid tissue damage has been observed). Epidemiological and clinical data show no evidence for immune suppression in vaccinated individuals.

5.2. Establishing and monitoring a national network. Monitoring quality of laboratory testing in regional virology laboratories of Kazakhstan

Dr Gaukhar Nusupbaeva, Kazakhstan, described activities for monitoring the virology laboratories in Kazakhstan. The Public Health service in the country has undergone frequent reorganizations, but is now back under the authority of the Ministry of Health (MoH). The NL works in collaboration with a 16 regional virology laboratories (SNLs) and is responsible for establishing and maintaining a national EQA programme, and providing organizational, methodological and practical support to the regional virology laboratories. The NL conducts serological testing, molecular testing (RT-PCR, genotyping), organization of the national EQA system (retesting; preparation of PT panels for regional laboratories), conducting assessment visits based on the results of EQA, and facilitating workshops and training sessions. The regional laboratories conduct serological testing of MR and CRS suspected patients, and seroprevalence studies. The regional laboratories also receive, check the condition, process and transport clinical samples for molecular testing in the NL.

The first EQA programme was launched in 2006 when the NL prepared PT panels for rubella. Currently regional labs do not conduct rubella testing themselves, all samples from suspected rubella cases being forwarded to the NRL, but they are still obliged to participate in EQA programme. In 2016 and 2017 all 16 laboratories scored 100% for both measles and rubella testing. In 2018 the NL started to use lyophilized panels and from 2019 only lyophilized PT panels will be distributed.

In 2017 the NL received ISO 17043:2013 accreditation that specifies general requirements for the competence of providers of proficiency testing schemes and for the development and operation of proficiency testing schemes. In 2018 the NRL received ISO 15189:2006 accreditation for medical laboratories. One challenge faced is that only Vector-Best (Novosibirsk, Russia) kits are available and the NL is unable to test PT panels with other assays. There are also difficulties in receiving some reagents from

Moscow via the courier service. No commercial in-house control panels are available in Kazakhstan and there is a lack of positive rubella samples. From 2015 to 2018 the RRL Moscow has provided the NL with in-house control panels and with PCR primers, conducted training on PCR and sequencing in Almaty and at the RRL, and there is on-going consultation with NL specialists.

Establishing and monitoring a national network: laboratory-based surveillance of measles and rubella in Italy

Dr Fabio Magurano, Italy, described the establishment of a laboratory network monitoring system in Italy. Italy is administratively divided into 21 regions with 21 regional health authorities, with a total of 146 local health units. In March 2015 a WHO EURO delegation, together with representatives from the RVC, delivered a number of policy and technical recommendations to the MoH. These recommendations included improving the integrated surveillance for measles and rubella and establishment of a national network of proficient diagnostic laboratories meeting the requirements of WHO. In March 2016 the Italian government financed the creation of MoRoNET – a network of SNLs coordinated by WHO NL. The was sent to the regions by the MoH requesting them to identify a local SNL for diagnosis and genotyping of measles and rubella cases or, alternatively, to send samples to the NL. 11 SNLs, in 10 regions, were initially identified and enrolled in the project, and a distance e-learning course addressed to health care workers with particular focus on the importance of laboratory diagnostics was prepared and organized. In 2017 another course was organized, and 4,000 health care workers passed the final test. A website[4] was developed to share news and information on MoRoNET and to exchange data between SNLs and the NL. Following the WHO scheme for annual accreditation, the NL organized a national serologic PT and molecular EQA, confirmatory testing and checklists for completion by the SNLs. The serologic PT panel , made up of 8 positive and 12 negative sera, is distributed annually. The molecular EQA is prepared using FTA Micro Cards and consists of 3 strains from the MeV strain bank of the NL together with 1 negative sample. The annual WHO accreditation checklist was translated into Italian and sent to each SNL, to be returned completed within 30 days. Evaluation reports have been sent to each SNL to communicate results of serologic PT and molecular EQA, together with an accreditation certificate. At present 14 SNLs in 13 of 21 regions are participating. SNLs are requested to share data with the NL on a monthly basis by completing 2 specific forms: one for sample testing results, and the second for identified sequences. A web-accessible database was developed and is used as a tool to share measles sequences and data between each SNL and the NL.

In 2017 Italy experienced a large measles outbreak with 5,042 measles cases reported. From January to September 2018 an additional 2,295 cases have been reported. MoRoNET was used to effectively manage the outbreak in 2017 and achieve the required WHO indicator for rate of laboratory investigations. Cases tested in proficient laboratories in 2017 reached 92%, compared to 16% in 2016. Moreover, from 2017 to date, the MoRoNET has allowed reporting of a large amount of sequence data to the WHO MeaNS database. Future plans include maintaining the standards achieved, to achieve an 80% rate of genotyping of chains of transmission, to train surveillance personnel, to draft an updated national elimination plan, and to organize a meeting of MoRoNet in April 2019.

[4] www.moronetlab.it

Discussion

- MoRoNET has accreditation at 3 levels: serology, molecular detection, and for all three components (serology, molecular detection and genotyping).
- It was decided that each SNL should submit sequences to MeaNS to speed up reporting times. Regions that do not have an SNL, refer samples to NL.
- WHO has been encouraging NLs to establish national networks and the countries themselves have been developing their own networks and EQA programmes following guidance from WHO. In RF and Turkey, the SNLs are officially considered as WHO SNLs, but WHO is not currently considering adopting SNLs in Italy and Kazakhstan as official WHO SNLs.

5.3. Laboratory contribution to annual NVC report.

German experience

Dr Sabine Santibanez, RKI Berlin, described the experience in Germany in providing the laboratory contribution to the annual NVC report. It was strongly recommended use be made of the new updated version of Vaccine-Preventable Diseases Surveillance Standards which was released in October 2018 by WHO HQ and is available on its website. The annual status update (ASU) in Germany is prepared by the national epidemiologist (Epi Manager) and national reference laboratory (NL), and then forwarded to the NVC. The NVC decides on the measles and rubella elimination status and forwards the ASU and NVC conclusion to the RVC. The RVC reviews the ASU and makes a final conclusion on the status. The role of the NL is to prepare a short summary description on the molecular surveillance which contains:

- An assessment on the elimination status, from a laboratory perspective, using laboratory data;
- The number and proportion of laboratory-investigated clinically-suspected, laboratory-confirmed and genotyped acute measles cases;
- Identification of the predominant MeV genotypes and sequence variants in the N450 region by genotype and distinct sequence ID from MeaNS, e.g. B3-4299 or D8-4807 (name strain can also be added);
- A short molecular-epidemiological characterization specified for the predominant MeV sequence variants. In Germany approximately 20 variants are seen each year and it is not felt necessary to describe each variant. Information on variants is provided as the number of detections, duration from week x to y, import/export events, virus spread across the country, specific characteristics of outbreaks, e.g. vaccine failure.
- If available MF-NCR data is included, particularly if interpretation is relevant for assessing the elimination status.

Visualization of molecular-epidemiological data provides information on the geographic and temporal distribution of detections of the predominant MeV sequence variants in detail for the year of reporting. In Germany the geographical and temporal distribution of cases in geopolitical subunits, such as federal states, is used.

Russian experience

Dr Sergey Shulga, RRL, Moscow, described the Russian experience of contributing laboratory data to the annual NVC report. The ASU is prepared by specialists of the National Centre for Measles and Rubella Surveillance (National Centre) who complete the report with a preliminary analysis of data and preliminary

conclusion and forwards it to the NVC. Following a series of meetings, the NVC experts either agree with the preliminary conclusions and that the preliminary data is sufficient, or raise questions and request additional information. This process takes several weeks, and eventually the ASU is finalized for sending to the RVC. Data collection and analysis continues throughout the year.

In the RF, in addition to laboratory testing of suspected measles and rubella cases, rash and fever cases are also tested. In accordance with national protocols, laboratory confirmation is performed on all measles and rubella cases that match the WHO case definitions, and on ≥2 cases of rash and fever cases per 100,000 population. Every territory must comply with this indicator and active search is usually conducted in infectious diseases hospitals and sometimes in non-infectious diseases clinics. Specimens from all suspected clinical cases are tested for both diseases in sequence. Th RF experience shows that in case of low measles/rubella incidence, if testing is conducted only on suspected measles and rubella cases, it is not possible to achieve the required surveillance indicator for discarded cases. Testing cases with rash and fever makes it possible to meet the discarded cases requirement. Furthermore, when a measles outbreak starts in territories that have not experienced measles for several years, the first cases are often detected through rash and fever surveillance. Through early recognition of such cases, the surveillance system can rapidly initiate response measures to prevent spreading. Even in territories with a relatively high incidence of measles, investigation of rash and fever cases continues, and among the rash and fever cases measles cases are also identified.

Implementation of MeV genotyping began in the RF in 2003, and RuV genotyping began in 2008, enabling a good baseline of data on circulation of genetic variants. Specialists at the National Center have developed national regulations for monitoring MeV and RuV circulation. These include key recommendations for regional centers of measles and rubella surveillance for genotyping at least 5 cases from each outbreak, and at least 3 epi-linked cases monthly for an ongoing outbreak; genotyping all clusters not epi-linked with outbreaks; genotyping all index cases if possible; genotyping all imported cases (alternatively – epi-linked cases); genotyping all “imported” within-country cases (alternatively – epi-linked cases); genotyping all sporadic cases from territories with sporadic incidence. Specimens in the regions are collected and sent to the laboratory of the National Centre, but genotyping is not immediately performed on all specimens received. The laboratory may conduct retrospective genotyping on specimens that are shown to be of epidemiological importance but not yet sequenced. This enables the laboratory to meet the WHO requirement of genotyping ≥80% of chains of transmission and have a comprehensive picture of virus circulation. Tables of circulation of MeV variants by week and by territory are updated throughout the year and then submitted to the NVC and subsequently to the RVC. This provides both temporal and geographical pictures of MeV circulation in the RF. A national database of case-based data (clinical, epi, laboratory incl. genotyping) of suspected measles, rubella and CRS cases has been developed and although not yet fully operational, it is in use.

Discussion

- All countries, including those that have eliminated measles and rubella, are required to document that they have good surveillance, that they are discarding an appropriate number of suspected cases, and that those discarded cases are geographically spread and not concentrated in the capital city where it is easiest to obtain specimens. Laboratories have the responsibility to collect that data and to show how much testing has been done, and that laboratory results are negative. Low

incidence countries should have a priority to focus on the quality of the surveillance system to be maintained despite absence of the diseases. It has been suggested for the next meeting to have presentations on laboratory input to the verification process in low incidence countries.

- The WHO secretariat reviews the reports and sometimes requests clarification from the laboratories if there is an issue or something is difficult to understand. There is a wide range of creativity from the laboratories and epidemiologists how data is being displayed in the ASUs. Germany and the RF are examples where there is a large amount of data presented in terms of outbreaks and cases, but there are also very explicit visual presentations from other countries, such as phylogenetic trees and epi curves. Each ASU contributing stake holder, particularly the laboratories, need to decide on the best way to present the data to make the decision of the RVC more straightforward.

5.4. Getting rubella sequences in elimination settings

Israel

Dr Vicki Indenbaum, Israel, described experience in Israel in obtaining rubella sequence data in elimination settings. Both endemic measles and rubella have been eliminated in Israel, and while the country experienced an import-related measles outbreak in 2018, only 3 rubella cases have been reported in the past 3 years. The surveillance system functions well, with each clinically suspected case immediately reported to one of the Health District Offices located all over the country. Samples for serology testing are sent to MoH licensed laboratories, which are hospital or private laboratories. Urgent samples and molecular samples are sent to the NL. After testing the licensed laboratories are required to refer IgM-positive samples to the NL for confirmation. A total of 3 rubella cases were registered from 2016 to 2018, with all 3 being unvaccinated males of approximately 40 years of age. According to epidemiological investigation, the case in 2016 was probably imported from the Czech Republic, in 2017 from Spain, and the case in 2018 was a taxi driver at the international airport. Samples from 2 cases were sent for measles investigation, and one for rubella testing. Several samples were collected from each case: serum, NP swabs and urine. Real time PCR was positive in all cases and only one case, in 2016, was IgM positive. Rubella genotype 2B was identified in all cases.

Every clinically suspected rubella or measles case is examined routinely for both diseases. When applicable, patients clinically suspected of Dengue or HHV-6 and cases of rash and fever are also examined for measles and rubella. The NL has developed an algorithm of laboratory investigation of suspected cases which depends on the sample collected. If the NL receives a serum sample, it is tested for both diseases in sequence. Molecular samples are tested by Real-time Multiplex PCR containing primers and probes for measles and rubella. The guidelines for collecting and storing molecular samples have been sent to clinicians and the laboratories.

Austria

Dr Heidemarie Holzmann, Austria, described experience in Austria in obtaining rubella sequence data. A single rubella case was reported in 2015, 3 cases in 2016, but in 2017 Austria faced two rubella outbreaks with a total of 39 cases. The first outbreak, with 21 cases (19 laboratory-confirmed and 2 epi-linked) occurred between February and April 2017 in an anthroposophical school in Vienna. The primary case was a school staff member suspected as measles but found to be positive for rubella following testing of the measles-negative sample for rubella IgM. Often in anthroposophical communities, individuals refuse

vaccination, but are prepared to be tested for evidence of infection. On testing, most were found to be negative for rubella IgG and IgM, but positive by PCR, and early rubella virus infection was. All cases with rash were also tested and RuV was detected in serum, OF or urine samples very early after onset of disease. Genotype 2B was identified in some of the samples.

The second rubella outbreak occurred between October 2017 and January 2018 with 19 cases detected in Upper Austria in a region where vaccine hesitancy is common. The primary case was a 34-year-old non-vaccinated male linked to importation from Bali. The case was suspected as measles and reported through the electronic reporting system. The NL received samples from the local laboratory for confirmation within 1 to 2 days and PCR for rubella was positive. Genotype 1E was identified in some of the samples. Authorities screened vaccination cards of all case contacts and vaccination was offered, but often not accepted.

Besides the two rubella outbreaks there was one case of rubella infection in pregnancy. Infection was imported from South Africa and diagnosed in early pregnancy by PCR. The patient was informed of the high risk of viral transmission to the fetus but the pregnancy was continued. The child was delivered in 2018 with a full picture of Gregg's syndrome plus additional cerebral abnormalities. The newborn shed virus for more than 4 months. Genotype 2B, but different from the genotype 2B of the outbreak (96% sequence identity), was detected in samples from both mother and child.

In 2017/18 a total of 27 rubella cases were detected by PCR, and in 18 of them the genotype was identified. 10 sequences were submitted to RubeNS.

Belarus

Dr Elena Samoilovich, Belarus, described the experience in Belarus. Investigation of RuV circulation in Belarus began in 2004, at which time the incidence of rubella was quite high (25 to 45/100,000 population), and every year 2,500 to 4,500 rubella cases were reported. In collaboration with the Luxemburg laboratory, specimens from 70 patients from different regions of the country were investigated using a protocol developed by Dr Judith Hübschen. 29 RuVs were isolated in Vero cells and sequences of the E1 gene (739nt molecular window) were obtained from cultural fluid. 2 RuVs from CRS cases were sequenced directly from clinical specimens (665nt and 300nt fragments were obtained). Phylogenetic analysis showed the RuVs belonged to 3 different genotypes of clade 1: 18 of 1H genotype, 7 of 1E and 6 of 1G. Two of the Belarus strains became reference strains (1H and 1G). After introduction of MMR and targeted SIAs between November 2005 and May 2006, rubella incidence decreased dramatically. After the decrease it became much more difficult to monitor for circulation of RuV, and most of the single cases detected between 2007 and 2013 were confirmed serologically. There were attempts at virologic investigation, but few cases were hospitalized. In addition, specimens often reached the laboratory after delay, and although in some cases the laboratory succeeded in amplifying RuV, the material was not good enough for genotyping. In 2015 the Moscow RRL provided the NL with training in molecular methods using a new protocol for detection and genotyping developed by CDC. Between 2014 and 2018, 5 cases of rubella were detected in Belarus, all importations. RuVs from all 5 cases were successfully genotyped. One RuV, imported from Indonesia, belonged to genotype 1E, and other four belonged to genotype 2B.

Based on the Belarus experience it was concluded that countries should have regulatory documents defining the procedure for virological examination of patients suspected of rubella, including the type of

material, timing of collection, etc. Close cooperation between the laboratories and epidemiologists/clinicians is essential. Introduction of a new MeV/RuV diagnostic and genotyping protocol in 2015, as well as the provision with primers developed by CDC have been of great benefit. Implementation of a nationally-funded research project aimed at improving the diagnosis of measles and rubella in the Republic of Belarus has also been very helpful. It is important to collect and investigate both NP swabs and urine. Of 35 RuVs successfully genotyped, 25 were from NP swabs, with 10 from urine. Samples should be collected on the first day after rash development and delivered to the laboratory preferably on the day of collection, but always within 48 hours. The material must not be frozen.

Discussion

- Questions were raised over reporting rubella test result in Israel when specimens are referred by clinicians only for measles testing. Rubella negatives are not reported in Israel, but positives are reported to the clinician and epidemiologists. The Epi department in Jerusalem is aware that the NL does parallel testing and does not routinely report negative rubella results to clinicians.
- In Austria, Israel and Belarus rubella is a notifiable disease.
- Because rubella is an asymptomatic or mild disease, contact tracing in outbreaks is very important. Many additional asymptomatic cases were detected in Austria through contact tracing.
- There are a number of commercial laboratories conducting rubella testing in Belarus. They are not accountable to NL, but all the laboratories are required to report IgM positive results in pregnant women and are obliged to refer these women to the NL for re-investigation. In the past five years all IgM positive results in pregnant women have been false-positives. It is difficult to investigate such cases, and the NL attempts to test for IgG, to amplify RuV, and establish dynamic observation. The number of such investigations has significantly decreased, but has not completely stopped.

Session 6A - Update on eLearning course development

Chair: Kevin Brown (GSL, London)

Myriam Ben Mamou, WHO regional office for Europe, described the eLearning project. While recognizing that face-to-face meetings and hands-on training are very useful, with the increasing size and diversity of the EU MR LabNet, there are severe challenges to providing conventional laboratory workshops and onsite laboratory training sessions. Development of an eLearning course could address some of the challenges, and there is an opportunity to use the recently revised laboratory manual for MR laboratory investigations as a basis for this course. The main objective of eLearning is to strengthen the skills of laboratory staff and keep their knowledge up-to-date, to encourage better performance and an improved contribution to high-quality case-based surveillance. The aim of the course is to be a one-stop shop for laboratory workers where they can find all of the information required to keep their knowledge updated and compliant with MR LabNet standards. There are many resources already available in the network, including previous training materials, that will be used as much as possible, together with material people would like to share.

Gaining approval of the eLearning course has been a long process. Draft terms of reference were developed in 2016, endorsement by the GMRLN the EUR RRLs was obtained in 2017. Internal consultations in EURO with other units (human resources, procurement) and also with WHO HQ, on how to better address the project, were held. It was decided to contract a commercial company to develop the product and requests

for proposals were sent out in May-June 2018. A selection panel composed of representatives of the technical and procurement units of EURO and capacity building officer from HQ has evaluated the offers and selected an appropriate supplier in the International Training Centre of the ILO (International Labor Organization). Phases of the project, as agreed in the request for proposals, are as follows:

> **Phase 1**: Situation analysis: analyzing training needs, available resources and providing expertise for options of the platform to be used for this course,
>
> **Phase 2**: eLearning package development after the decision has been made as to which platform to choose,
>
> **Phase 3**: Transfer to online e-learning platform conduct testing.

To guide this project an eLearning Advisory Committee was established in April 2017 at the EUR RRL meeting in Moscow. The Committee has been formed of representatives from RRLs, the GSL, and also from two NLs (Belarus and Denmark). The committee is expected to validate the deliverables of each phase.

The first 4 priority modules will be:

1. Introductory module to the MR LabNet and eLearning Course, including introduction to the new WHO Laboratory manual.
2. Module on EIA, in particular preparation of internal control, use and interpretation.
3. Module on genotyping and sequence management.
4. Interpretation of laboratory results for case classification.

The course is modular and allows staff to take whichever module that they need for their practice. In future further modules will be developed.

Update on project development

Alessia Messuti and Fatma Feki of the Learning Innovation Team of the International Training Centre of the International Labor Organization (ITCILO) provided an update on project development. The project aim is to have an online platform accessible by all staff members of the EUR MR LabNet, initially offering the 4 modules (see above). The development team will create a course that will be not only human-centered, but also very adaptive to each member of the LabNet. All solutions must be sustainable, easily adaptable, easily editable, easily updated and available anytime, anywhere, and also engaging. The team will be using presentations and Webinars to incorporate them into the platform. Learners will be able to connect with colleagues across the network for discussion, and explore collaborations online. The team is following the philosophy of *Design Thinking*, a process used in many sectors including industrial design, but increasingly in education. Initially, the team needs to understand the audience, the challenges workers of the LabNet are facing and how they normally conduct their work. The team then needs to explore the options for the type of learning experience required. The draft course will be tested by an eLearning Advisory Committee. One strategy is to use technology to create an adaptive learning process where a learner can pause and come back to a task at the learner's own pace.

The team started with a user research survey to understand how people work in the laboratory, including the challenges and level of collaboration. 81 responses were collected (61 from English speaking and 20 - Russian), almost half of responders were heads of the laboratories. Collaboration is the main way of

working in the laboratory (95%), and 84% said they needed training, resources and equipment; of these 40% said that training is a priority. From the data collected, the team will create "empathy maps", categorizing users, and attempt to define the profile of the end audience (head of NL, head of SNL, junior biologist, lab technician, etc.). The team has also investigated common laboratory procedures, reducing these to common tasks that the different learners can complete. Every profile will have a different learning path, depending on the learner's functional responsibilities in the laboratory. The team is close to finalizing the first phase of development and will soon move on to the task analysis phase. For each procedure the team will develop task situations, with exercises for each task, e.g. there is a sequence management module tasks in the platform that will be available for learners to take a step-by-step guided exercise on how to use the software and analyze sequences. Each exercise will be validated by the eLearning Advisory Committee. The team is currently exploring the 2 platforms that will host the training, these are the Agora and OpenWHO platforms. Testing and implementation of the course is scheduled for the end of February and will be conducted in English. Translation into other WHO languages will follow.

3. Summary and recommendations

The following recommendations result from the exchanges and discussions between the participants throughout both meetings (Western and Central European countries, 13-15 November 2018, and Russian Federation and Newly Independent States, 14-16 November 2018)

Serology

1. Many countries do not have rubella IgM in-house control; absence of this control reduces score of serology PT. Laboratories that use commercial options are asked to share information with Regional Laboratory Coordinator for dissemination.
2. EURO has switched to Euroimmun ELISA kit due to planned interruption of DiaSorin Siemens production. Regarding its use with DBS, DSS:
    - DBS evaluation was done by the manufacturer who validated the use of Euroimmun IgM with DBS on PerkinElmer paper. RRLs are encouraged to investigate if similar results are obtained with Whatman paper or if it is needed to use only PerkinElmer paper.
    - Evaluation for dried sera spots (DSS) by RRL Luxemburg has shown insufficient stability after 1-2 weeks of storage at 30^0C temperature. RRL Luxemburg will try adding a stabilizing agent and will continue the evaluation. RRL Moscow and RRL Luxemburg will collaborate on a harmonized protocol. NIS Labs should continue sending DSS for retesting to their RRL (retesting will be done with Euroimmun).
3. For Euroimmun IgM ELISA kit labs should use the semi-quantitative method of calculation of the result: ratio (sample OD/calibrator OD); result interpretation: <0.8 negative, ≥0.8…≤1.1 equivocal, ≥1.1 positive. Labs should submit information accordingly when reporting serology PT results online with all the required data.
4. Labs are invited to share their data of EIA comparison studies (non-inferiority) with Regional and Global Coordinators.
5. WHO will disseminate the results of kits' comparison study currently been prepared by HQ. Evaluation of 4-5 different brands of kits for IgM and IgG will be conducted by Public Health Agency,

Canada (coordinated by HQ). Labs which have collections of interesting sera (well characterized, serology, PCR) are invited to donate to WHO for these studies.

6. Microimmune users should use the most recent version of the instructions (English insert is the most updated) and make sure the OF diluent contains fetal calf serum.

Molecular surveillance

7. Laboratories are encouraged to facilitate collection of virologic specimens to ensure having representative sequences of sporadic cases and outbreaks for measles and rubella. Laboratories should send samples or PCR products to RRLs or GSL if no sequencing capacities exist in the National lab.
8. If virologic specimens are not available, laboratories should use alternative specimens like whole blood, DBS or sera from sporadic cases for measles and even for rubella for sequencing.
9. RRL Luxemburg will share protocol for PCR and sequencing of serum samples.

10. Laboratories are asked to share well-working protocols for WGS and MF-NCR with the network through the laboratory manual.
11. Laboratories are reminded not to overinterpret the data from MeaNS and RubeNS and also to comply with the Terms & Conditions of use of these databases.
12. MF-NCR and WGS may offer greater resolution for countries in elimination settings, further guidance will come from WHO NEW Working Group.
13. Only NIBSC or US CDC controls in molecular tests should be used, vaccine strain cannot be used. WHO will facilitate obtaining these controls (to ease shipping).
14. Labs are reminded to share sequence data, and in timely manner; labs that have sequences and have not shared please submit to MeaNS.
15. Labs should apply high standards to sequence analysis. Sequences of bad quality should not be used.
16. WHO naming of sequences: labs should use only standard ASCII characters in the name (no special characters), and pay attention to the location used (sometimes difficult to find). Steering Committee decisions will come. Some constrains will be put on the naming convention in a new MeaNS/RubeNS version.
17. Labs submitting sequences to MeaNS / RubeNS need to be proficient (should have passed mEQA for sequencing).
18. When a new named strain is identified in MeaNS it will appear (with a date) on a pop-up message when user logs on into MeaNS.

Molecular EQA

19. EURO has switched to the WHO/CDC US global mEQA programme like all other WHO regions. Participating labs should send result forms and supporting data to CDC GSL email addresses within 6 weeks of panels reception (additional information is provided in mEQA slides presented during the meeting by US CDC GSL).
20. In case of issue, CDC GSL will offer retest if the problem can be resolved by repeating a test.

Accreditation

21. Accreditation check-list is being revised for next round, including the addition of a 10th essential criteria: when applicable, confirmatory test results of SubNational Laboratories' referred specimens are reported within 14 days for ≥ 80% of specimens received.
22. For accreditation round 2019 (data of 2018) laboratories should fully complete Part 2 of accreditation checklists with their self-assessment as part of the annual review.
23. For accreditation round 2020 (data of 2019) minimum number of molecular testing for measles and for rubella will be required which is 2 tests per quarter.
24. Regarding SubNational networks in Kazakhstan and Italy LabNet members are asked to send to Regional and Global Coordinators their comments on potentially adopting SNLs as part of WHO MR LabNet.

Verification

25. Rate of discarded cases is critical for the verification. It is important that sensitivity of surveillance should be documented at subnational level as well.
26. MeaNS / RubeNS distinct sequence ID are required in the ASU report.
27. All 2018 sequences should be reported to MeaNS and RubeNS in time to the next ASU report (due 15st of April 2019).

Surveillance

28. Network laboratories should use recommendations and protocols of the Measles and Rubella Laboratory Manual, 3rd edition, 2018 (available online at http://www.who.int/immunization/monitoring_surveillance/burden/laboratory/manual/en/ ; the Russian-language version will be available soon), and the October 2018 version of Vaccine Preventable Diseases Surveillance Standards (available online at http://www.who.int/immunization/monitoring_surveillance/burden/vpd/standards/en) as well as recommendations and testing algorithm proposed by the RRL.
29. Measles reinfection cases should be reported, LabNet should use the lab algorithms of the Lab Manual. Further guidance to be solicited from WHO / SAGE MR working group on how to report these cases, particularly for the diagnostic approach and communication aspects.
30. WHO will work together with ECDC to align the definition of measles reinfection case.
31. Labs are recommended to apply WHO guidance regarding serology and sequencing testing in outbreak situation: WHO does not recommend epi-linked cases to be tested to preserve laboratories' resources (please refer to WHO VPD Surveillance Guidelines).
32. Collaboration between laboratories and epidemiologists is essential and should be strengthened. The labs should interpret lab results for epidemiologists periodically; laboratory results cannot answer all questions.

Training

33. EURO to strengthen the use of eLearning resources (webex, eLearning modules) and to keep HQ informed on progress of eLearning course development.

Additional recommendations were developed by participants from the WHO European Regional Measles/Rubella LabNet Meeting for Russian Federation and Newly Independent States, 14-16 November 2018 :

34. RRL to provide all laboratories with Excel program for analyzing and interpreting testing results with Euroimmune kit. Use of the program is up to laboratories;
35. To ensure the quality of testing with Euroimmune kit, continue to use the in-house laboratory controls provided by the RRL earlier, according to the recommended protocol. Aliquots of the working dilution are stable when stored at -20 C for at least 3 years;
36. RRL to ensure regular re-testing of the expired in-house controls, extending the expiration date and informing the laboratories about the extension of the expiration date;
37. Network laboratories to provide RRL in-house control charts for 2018 for data analysis and development of additional recommendations on the use of the controls (if necessary);
38. Network laboratories to search the possibility of obtaining diagnostic kits for measles/rubella on the local market for IgG testing, antibody avidity;
39. RRL and EURO consider the possibility of providing the necessary EIA test kits (IgG, antibody avidity) if not possible to purchase them in the local market;
40. RRL to provide laboratories with recommendations on the choice of measles / rubella EIA diagnostic kits for IgG detection and antibody avidity;
41. Laboratory staff participating in the molecular EQA in connection with the transition from using INSTAND panels to CDC panels before testing the panel, should carefully read the presentation of Dr. B. Bankamp and apply the panel reporting instructions
42. Laboratories planning the introduction of molecular testing to analyze the need for such testing, the availability of samples, resources (equipment, reagents, personnel), a list of tests and methods planned for implementation. Provide to RRL implementation plan and application for training specialists;
43. RLL to hold in 2019 training for specialists in molecular methods on the requests of the laboratories;
44. RRL to send (if necessary) in 2019 specialists to provide on-site methodological assistance in the implementation of molecular methods;
45. Continue sending to RRL samples (swabs + urine) from patients with measles and rubella on FTA paper (Whatman® FTA® Elute, Indicating FTA Micro Card with sample area per card, WHAWB120412 / WHAWB120411) for virus genotyping. Original samples should be collected in accordance with the previously submitted protocol and stored at -80° C for at least 12 months after receipt.

4. Annex

List of Participants

Albania

Alma Robo
Responsible for Measles/Rubella, Laboratory of Virology
Institute of Public Health

Armenia

Shushan Sargsyan
Head, Virology Laboratory
Republican Centre for Disease Control and Prevention

Austria

Heidemarie Holzmann
Professor, Institute of Virology
Medical University of Vienna

Azerbaijan

Nazim Shugayev
Head, Laboratory of Immunology
Republican Anti-Plague Station

Belarus

Elena O. Samoilovich
Head, Laboratory of Vaccine-preventable Diseases
Republican Research and Practical Center for Epidemiology and Microbiology

Belgium

Veronik Hutse
Head of National Laboratory, Section of Virology, Department of Microbiology
Scientific Institute of Pubilc Health

Bosnia and Herzegovina

Amela Dedeic-Ljubovic
Head, Clinical Microbiology Laboratory
University of Sarajevo

Pava Dimitrijevic
Head, Department of Microbiology
Public Health Institute of the Republic of Srpska

Bulgaria

Stefka Krumova Ivanova
Head of Laboratory, Department of Virology
National Center of Infectious and Parasitic Diseases

Croatia

Jelena Ivancic Jelecki
Scientific Associate, Centre for research and knowledge transfer in biotechnology
University of Zagreb

Cyprus

Panayiota Hadjikyprianou
Head, Immunology Laboratory
Nicosia General Hospital

Czech Republic

Radomira Limberkova
Measles and Rubella Laboratory, Head
National Institute of Public Health

Denmark

Lasse Dam Rasmussen
Virologist, Department for Microbiological Diagnostics and Virology
Statens Serum Institut

Jesper Rønn
Laboratory Technician
Department for Microbiological Diagnostics and Virology

Estonia

Grete-Katariine Kuum
Head, Measles Rubella Laboratory
Health Board

Finland

Anu Haveri
Researcher, Infectious Disease Control and Vaccinations, Department of Health Security
National Institute for Health and Welfare

Mia Kontio
Senior specialist, Department of Health Security
National Institute for Health and Welfare

France

Julia Dina
Laboratory of Human and Molecular Virology
University Hospital of Caen

Georgia

Nazibrola Chitadze
Head of Serology Laboratory, Department of Virology, Molecular Biology and Genome Research
National Center for Disease Control and Public Health (NCDC)

Germany

Sabine Santibanez
Virologist, WHO Measles Rubella Regional Reference Laboratory
Robert Koch Institute

Greece

Elina Horefti
Technical Supervisor, Measles and Rubella National Reference Laboratory
Hellenic Pasteur Institute

Ireland

Jeff Connell
Deputy Director, National Virus Reference Laboratory
University College Dublin

Linda Dunford
National Virus Reference Laboratory
University College Dublin

Israel

Victoria Indenbaum
Director, National Centre on Measles, Rubella and Mumps
Chaim Sheba Medical Centre

Italy

Fabio Magurano
Virologist, Infectious Parasitic and Immuno Mediated Diseases
Istituto Superiore di Sanita

Loredana Nicoletti
Head, Laboratory of Infectious Parasitic and Immuno Mediated Diseases
Istituto Superiore di Sanita

Kazakhstan

Gaukhar Nusupbayeva
Head, National Reference Laboratory for Control of Viral Infections
Scientific and Practical Center of Sanitary and Epidemiological Expertise and Monitoring

Latvia

Tatjana Kolupajeva
Head, Viral Infections Molecular Biology Division
Riga Eastern University Hospital

Lithuania

Vilnele Lipnickiene
Virologist, Laboratory Medicine Physician
National Public Health Surveillance Laboratory

Luxembourg

Judith Hübschen
Virologist, Department of Infection and Immunity
Luxembourg Institute of Health

Malta

Robert Decelis
Senior Medical Laboratory Scientist, Virology Laboratory
Mater Dei Hospital

Montenegro

Željka Zeković
Centre for Medical Microbiology

Netherlands

Rogier Bodewes
Head, Measles Rubella Laboratory
National Institute for Public Health and the Environment

Rik L. de Swart
Senior Scientist, Department of Virology
Erasmus Medical Centre

North Macedonia

Golubinka Bosevska
Head, Laboratory for Virology and Molecular Diagnostics
Public Health Institute

Norway

Jennifer Dembinski
Norwegian Institute of Public Health

Susanne Gjeruldsen Dudman
Head of National Polio Laboratory
Norwegian Institute of Public Health

Poland

Agata Makówka
National Institute of Public Health

Portugal

Paula Palminha
Head, Vaccine-preventable Diseases Reference Laboratory
National Institute of Health Dr Ricardo Jorge

Republic of Moldova

Alina Cataraga
Head, Reference Laboratory in Microbiology
National Agency for Public Health

Romania

Mihaela Lazar
Head of Laboratory, Respiratory Viral Infections
"Cantacuzino" National Medico-Military Institute for Research and Development

Russian Federation

Tamara Mamaeva
Virologist, Measles/Rubella Regional Reference Laboratory
G.N. Gabrichevsky Research Institute for Epidemiology and Microbiology

Sergey Shulga
Head, Measles/Rubella Regional Reference Laboratory
G.N. Gabrichevsky Research Institute for Epidemiology and Microbiology

Serbia

Maja Bančević
Head, National Measles and Rubella Laboratory
Institute of Virology, Vaccines and Sera "Torlak"

Slovakia

Alexandra Polčičová
Head of Laboratory, National Reference Center for Measles, Rubella and Mumps
Public Health Authority

Slovenia

Katarina Prosenc Trilar
Head, Laboratory for Public Health Virology
National Laboratory of Health, Environment and Food

Spain

Aurora Fernández García
Head, Diagnostic Microbiology Division
Centro Nacional de Microbiología

Sweden

Katherina Zakikhany
Senior Microbiologist, Laboratory Surveillance of Vaccine-preventable Diseases
Public Health Agency of Sweden

Switzerland

Pascal Cherpillod
Head, Virology Laboratory
University Hospital of Geneva

Tajikistan

Farida Tishkova
Head, Virology Laboratory
Tajik Research Institute of Preventive Medicine

Turkmenistan

Amansoltan Ashyrova
Head, Viral Department
Central Reference Laboratory

Ukraine

Iryna Demchyshyna
Head, Virology Laboratory
Ministry of Health

United Kingdom of Grean Britain and Northern Ireland

Kevin Brown
Head, Immunisation and Diagnosis Unit, Virus Reference Department
Public Health England

Uzbekistan

Sultana Djemileva
Head of Virology Laboratory
Republican Center of State Sanitary Epidemiological Surveillance

Observer

Gjyle Mulliqi Osmani
Associate Professor, Department of Microbiology
Institute of Public Health, Pristina

International Training Centre of the International Labour Organization

Fatma Feki

Alessia Messuti

Regional Verification Committee

Irja Davidkin

European Centre for Disease Prevention and Control

Nick Bundle
Expert, Vaccine-preventable Diseases
European Centre for Disease Control and Prevention

World Health Organization

Headquarters

Mick Mulders
Scientist, Global VPD Laboratory Networks

Regional Office for Europe

Myriam Ben Mamou
Coordinator, European Regional Measles / Rubella Laboratory Network
Vaccine-Preventable Diseases and Immunization

Florencia Biviano

Programme Assistant, Vaccine-preventable Diseases and Immunization

Siddhartha Datta
Programme Manager, a.i., Vaccine-Preventable Diseases and Immunization

Shahin Huseynov
Technical Officer, Accelerated Disease Control, Vaccine-Preventable Diseases and Immunization

Dragan Jankovic
Technical Officer, Accelerated Disease Control, Vaccine-Preventable Diseases and Immunization

Mark Muscat
Technical Officer, Accelerated Disease Control, Vaccine-Preventable Diseases and Immunization

Patrick O'Connor
Team Lead, Accelerated Disease Control, Vaccine-Preventable Diseases and Immunization

Rapporteur

Dovile Videbaek

Interpreters

Olga Aleksinskaya

Vladimir Ilyukhin

The WHO Regional Office for Europe

The World Health Organization (WHO) is a specialized agency of the United Nations created in 1948 with the primary responsibility for international health matters and public health. The WHO Regional Office for Europe is one of six regional offices throughout the world, each with its own programme geared to the particular health conditions of the countries it serves.

Member States

Albania
Andorra
Armenia
Austria
Azerbaijan
Belarus
Belgium
Bosnia and Herzegovina
Bulgaria
Croatia
Cyprus
Czechia
Denmark
Estonia
Finland
France
Georgia
Germany
Greece
Hungary
Iceland
Ireland
Israel
Italy
Kazakhstan
Kyrgyzstan
Latvia
Lithuania
Luxembourg
Malta
Monaco
Montenegro
Netherlands
North Macedonia
Norway
Poland
Portugal
Republic of Moldova
Romania
Russian Federation
San Marino
Serbia
Slovakia
Slovenia
Spain
Sweden
Switzerland
Tajikistan
Turkey
Turkmenistan
Ukraine
United Kingdom
Uzbekistan

World Health Organization
Regional Office for Europe

UN City, Marmorvej 51, DK-2100 Copenhagen Ø, Denmark
Tel: +45 45 33 70 00 Fax: +45 45 33 70 01
Email: eucontact@who.int
Website: www.euro.who.int