Lessons Learned/Examples from the Molecular Epidemiology of Measles

Paul Rota*

Centers for Disease Control and Prevention

Topic: Webinar on Molecular Genotyping for MR
Date: Tuesday, 5. December 2017

*The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.
Lessons Learned from > 20 years of Genetic Characterization of Measles Viruses

• All vaccines strains are in genotype A

• Wild-type viruses in genotype A no longer circulate

• Rapid confirmation of vaccine reactions is an important part of laboratory surveillance in elimination settings

• Sequence information allowed development of RT-PCR based assays for rapid confirmation of vaccines reactions
A measles vaccine PCR was developed and validated

A collaboration between:

- National Microbiology Laboratory (Canada)
- Centres for Diseases Control (USA)
- Robert Koch Institute (Germany)
Rapid Assay to Detect Measles Vaccine Strains

- MeVA allows confirmation of MR, MMR vaccine reactions in 3-4 hours compared to 24-48 for sequencing
- Validations in progress
Lessons Learned from > 20 years of Genetic Characterization of Measles Viruses

- Countries with endemic measles have multiple, co-circulating lineages of measles virus, endemic genotype or genotypes (e.g. genotype H1 in China, B3 in sub-Saharan Africa)
Endemic Measles/Endemic Genotypes
Lessons Learned from > 20 years of Genetic Characterization of Measles Viruses

• In countries that have eliminated measles, the pattern of genotypes reflects the sources of imported virus
  • Lack of endemic genotype is an essential criterion for verification of elimination
Measles Eliminated/Multiple Genotypes

Size of circle is proportional to the number of specimens with a genotype result.

- B3
- D8
- D3
- D9
- D4
- G3
- D6
- H1

Countries with genotype data available
Not applicable
Lessons Learned from > 20 years of Genetic Characterization of Measles Viruses

• In countries where measles has been reintroduced, often only a single lineage is detected; there may be a “switch” in genotypes indicating single source of importation and single chain of transmission

• Tracking by using named strains can help to identify sources

• Sequence data can help documents switch in genotypes after elimination and following reintroduction
Measles cases by year, Philippines, 2000-2014

2000-2004
Genotype D3 is the endemic genotype in the Philippines and D3 imports to other countries are traced to the Philippines

2004-2005
Measles campaign in 2004, no measles detected in 2005

2007-2009
Genotypes D9 and G3 introduced and detected in outbreaks

2014-2015
Genotype B3 introduced, genotype B3 and D9 continues to circulate and cause outbreaks

Genotype D3 not detected globally after 2004
Mapping Transmission Pathways Following the Outbreak in the Philippines in 2014: Tracking Genotype B3 “Harare”

Global transmission of measles viruses from the Philippines, 2014
Tracking Measles Importations by Named Strain (Lineage)
Tracking Measles Lineages by using Named Strains

Time in months

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A. Genotype B3
- In both cases, genotype B3 is reported every month for 1 consecutive months
- Example A, reporting only genotype: continuous transmission of genotype B3
- Example B, tracking named strains: Multiple importations of three different named strain/lineages

B. B3-name 1
B3-name 2
B3-name 3
Weekly distribution of measles genotype D8 lineages identified at VIDRL in 2016.

Country codes of importation associated with measles cases are indicated (IDN=Indonesia, VNM=Viet Nam; IND=India; NZL=New Zealand; NPL=Nepal; THA=Thailand)
Phylogenetic tree based on the N-450 gene sequences of measles viruses detected in the US in 2014. Reference strains are underlined and color code for the genotypes is given at bottom left.
Lineages of Measles Viruses Detected in the US during 2014. Strains from the US are listed in the left column. Strains with exact matches in N-450 are listed on the right column. Named lineages (from MeaNS) are shown in red. NEM refers to no exact match.

<table>
<thead>
<tr>
<th>Measles Representative Strain</th>
<th>Gen.</th>
<th>Number of Strains</th>
<th>MeaNS Exact Match</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVs/New York.USA/15.14/2</td>
<td>B3</td>
<td>+ 4 identical strains</td>
<td>NEM</td>
</tr>
<tr>
<td>MVs/Missouri.USA/22.14</td>
<td>B3</td>
<td>+ 1 identical strains</td>
<td>NEM</td>
</tr>
<tr>
<td>MVs/Michigan.USA/48.14/1</td>
<td>B3</td>
<td>+ 4 identical strains</td>
<td>NEM</td>
</tr>
<tr>
<td>MVs/Alaska.USA/37.14/ (CDC_COE-2014-172)</td>
<td>B3</td>
<td>NEM</td>
<td></td>
</tr>
<tr>
<td>MVs/California.USA/4.14/ (CDC_COE-2014-3)</td>
<td>B3</td>
<td>NEM</td>
<td></td>
</tr>
<tr>
<td>MVs/California.USA/6.14/1 (CDC_COE-2014-9)</td>
<td>B3</td>
<td>NEM</td>
<td></td>
</tr>
<tr>
<td>MVs/California.USA/8.14/3 (CDC_COE-2014-18)</td>
<td>B3</td>
<td>+ 2 identical strains</td>
<td>NEM</td>
</tr>
<tr>
<td>MVs/New York.USA/8.14/6 (CDC_COE-2014-24)</td>
<td>B3</td>
<td>NEM</td>
<td></td>
</tr>
<tr>
<td>MVs/California.USA/5.14/ (CDC_COE-2014-7)</td>
<td>B3</td>
<td>+ 81 identical strains MVs/Harare.ZWE/38.09/</td>
<td></td>
</tr>
<tr>
<td>MVs/Kosrae.FSM/21.14/2</td>
<td>B3</td>
<td>+ 69 identical strains MVs/Harare.ZWE/38.09/</td>
<td></td>
</tr>
<tr>
<td>MVs/Kosrae.FSM/21.14/4</td>
<td>B3</td>
<td>NEM</td>
<td></td>
</tr>
<tr>
<td>MVs/Pohnpei.FSM/28.14/2</td>
<td>B3</td>
<td>NEM</td>
<td></td>
</tr>
<tr>
<td>MVs/Chuuk.FSM/34.14</td>
<td>B3</td>
<td>NEM</td>
<td></td>
</tr>
<tr>
<td>MVs/Indiana.USA/30.14/</td>
<td>H1</td>
<td>+ 1 identical strains MVs/Liaoning.CHN/23.14/2, MVs/Tianjin.CHN/22.14/4, MVs/Shanghai.CHN/20.14/, MVs/Anhui.CHN/19.14/8</td>
<td></td>
</tr>
<tr>
<td>MVs/California.USA/1.14/ (CDC_COE-2014-23)</td>
<td>H1</td>
<td>MVs/Hong Kong.CHN/49.12</td>
<td></td>
</tr>
<tr>
<td>MVs/Texas.USA/36.14/ (CDC_COE-2014-171)</td>
<td>H1</td>
<td>MVs/Hong Kong.CHN/42.11/</td>
<td></td>
</tr>
<tr>
<td>MVs/Ohio.USA/28.14</td>
<td>D9</td>
<td>NEM</td>
<td></td>
</tr>
<tr>
<td>MVs/Ohio.USA/16.14/1</td>
<td>D9</td>
<td>+ 37 identical strains MVs/Hong Kong.CHN/08.14/2</td>
<td></td>
</tr>
<tr>
<td>MVs/Washington.USA/12.14/ (CDC_COE-2014-69)</td>
<td>D8</td>
<td>+ 2 identical strains MVs/Taunton.GBR/27.12/</td>
<td></td>
</tr>
<tr>
<td>MVs/California.USA/4.14/2 (CDC_COE-2014-11)</td>
<td>D8</td>
<td>NEM</td>
<td></td>
</tr>
<tr>
<td>MVs/California.USA/8.14/ (CDC_COE-2014-14)</td>
<td>D8</td>
<td>NEM</td>
<td></td>
</tr>
<tr>
<td>MVs/Hawaii.USA/43.14/3</td>
<td>D8</td>
<td>+ 2 identical strains MVs/Queensland.AUS/45.14/4, MVs/London.GBR/30.14, MVs/NewSouth Wales.AUS/27.14/, MVs/WesternAustralia.AUS/23.14/</td>
<td></td>
</tr>
<tr>
<td>MVs/Massachusetts.USA/14.14</td>
<td>D8</td>
<td>MVs/London.GBR/22.12/3, MVs/Victoria.AUS/6.11/ MVs/Ludwigsburg.DEU/13.10/</td>
<td></td>
</tr>
<tr>
<td>MVs/Ohio.USA/19.14</td>
<td>D8</td>
<td>MVs/Maastricht.NLD/14.14, MVs/Pune.IND/38.13 MVs/WesternAustralia.AUS/51.13</td>
<td></td>
</tr>
<tr>
<td>MVs/Massachusetts.USA/19.14</td>
<td>D8</td>
<td>MVs/Heidelberg.DEU/45.13/</td>
<td></td>
</tr>
<tr>
<td>MVs/California.USA/20.14/ (CDC_COE-2014-118)</td>
<td>D8</td>
<td>MVs/HuluLangat.MYS/26.11</td>
<td></td>
</tr>
<tr>
<td>MVs/California.USA/8.14/2 (CDC_COE-2014-17)</td>
<td>D8</td>
<td>MVs/FrankfurtMair.DEU/17.11/</td>
<td></td>
</tr>
<tr>
<td>MVs/California.USA/12.14/8 (CDC_COE-2014-90)</td>
<td>D8</td>
<td>+ 4 identical strains MVs/Western Australia.AUS/12.14/, MVs/London.GBR/9.14/2 MVs/Singapore.SGP/13.14/</td>
<td></td>
</tr>
</tbody>
</table>
MeV – Genotype B3 imported into the USA in 2015, multiple importations from a single source and spread to multiple states
Increasing the Resolution of Molecular Epidemiology for Measles

- WHO standard genotyping N target 450 nt
- MF-NCR new target 1018 nt
- Alternate genotyping target: H ORF, 1854 nt
- WGS-t 15,875 nt

- WHO N.E.W. Working Group to report in January
- Sequencing addition regions or the entire genome can increase resolution in some cases
Identical N450 (a) and H (b) gene target sequences

Courtesy: A Severini, NML, Canada
The MF-NCR (B) offers a resolution comparable to WGS (A)

Courtesy: A Severini, NML, Canada
Fig 3. Phylogenetic analysis of the N-450, H, N and M/F NCR sequences of D8 outbreak strains.

Genotype D9 from Single Importation into the USA 2014: Identical N-450 and MF-NCR Sequences
Completeness of Molecular Surveillance: USA 2001-2015

<table>
<thead>
<tr>
<th>Cases</th>
<th>Total</th>
<th>Genotyped</th>
<th>Total</th>
<th>Genotyped</th>
<th>Total</th>
<th>Genotyped</th>
<th>Total</th>
<th>Genotyped</th>
<th>Total</th>
<th>Genotyped</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016</td>
<td>509</td>
<td>238 (46%)</td>
<td>77</td>
<td>47 (63%)</td>
<td>61</td>
<td>47 (77%)</td>
<td>50</td>
<td>47 (96%)</td>
<td>748</td>
<td>523 (71%)</td>
</tr>
</tbody>
</table>
Summary

• Genetic characterization of measles virus by the GMRLN has made substantial contributions to understanding both the biology and evolution of measles viruses, and has become an integral part of routine laboratory surveillance for measles.

• The GMRLN needs to continue to build capacity for genetic characterization of both measles and rubella and to integrate new testing schemes and new technologies.
What RVC members should expect from GMRLN regarding molecular epidemiology of measles and rubella

- **Strategically** increasing capacity for molecular testing
  - Network laboratories with existing molecular infrastructure (RT-qPCR, sequencing) trained for measles and rubella molecular testing
    - Ability to analyze and upload quality sequences to MeaNS and RubeNS
  - Workshops will be conducted in as needed in all WHO Regions
  - Molecular external quality assurance program expanded to monitor the performance of laboratories
RVC Members should look for

- Laboratories must work with program staff to obtain adequate samples for viral detection (>80% of chains of transmission, with genotype)

- Obtain virologic samples from all cases and report genotypes even from sporadic cases
  - Timely reporting of all sequence data to MeaNS and RubeNS

- Implementation molecular methods for case classification/confirmation
  - RT-PCR
Continued…..

• Rapid confirmation of measles vaccine reactions is essential

• Description of the named strains/lineages in addition to the genotypes
  • Always include a phylogenetic tree
  • Always include matches with named strain or identical sequence on MeaNS/RubeNS
  • Consider using expanded sequencing windows to increase resolution when necessary
  • Consider additional training in molecular methods including sequence analysis and database submission (keep using webinars)
Questions??

Discussion