Two Recent Cases of Congenital Rubella in New York State

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Background

- Rubella, caused by a togavirus of the genus *Rubivirus,* has a ss (+) sense RNA genome and icosahedral capsid, surrounded by a lipid envelope with prominent spike proteins
- Children infected with rubella often develop few symptoms, but adults may experience 1-5 days of fever, headache, malaise, coryza and conjunctivitis
- However, rubella infection during pregnancy with fetal infection, especially during the first trimester, is likely to result in congenital rubella syndrome (CRS) with miscarriages, stillbirths and severe birth defects
- Although officially eliminated in the Western Hemisphere in April 2015, rubella remains endemic in many other regions of the world
- In 2012, 4 public health laboratories (PHLs) were selected as Vaccine Preventable Disease (VPD) Reference Centers:
  - Minnesota Public Health Laboratory, Wisconsin State Laboratory of Hygiene, California Department of Public Health Laboratory and the Wadsworth Center Virology Laboratory at the New York State Department of Health
- During the winter of 2013 and the summer of 2015, the Wadsworth Center Laboratory confirmed two suspected cases of CRS in New York State, both infants born to mothers returning from Yemen
- The purpose of this study was to confirm and genetically characterize the rubella virus in samples from those two infants with suspected CRS

Methods

- Nasopharyngeal swab (NPS), throat swab and urine samples collected from two infants in New York State on 12/13/2013 and 7/20/2015 were submitted to the Wadsworth Center for testing
- Nucleic acid was extracted and purified from the samples using a NucliSENS EasyMAG instrument
- Rubella virus RNA was detected using a TaqMan real-time RT-PCR assay provided by the Centers for Disease Control and Prevention as part of the Vaccine Preventable Diseases program and run on an ABI 7500 Fast Dx instrument
- Conventional RT-PCR was performed to amplify a 945 bp portion of the rubella E1 gene
  - Two primer sets generated overlapping 480 and 633 bp regions of the E1 gene
  - Qiagen Q Solution was added to the PCR reactions due to the extreme GC rich nature of the rubella genome
- PCR-amplified products were visualized on 1% TAE agarose gels and the amplicons purified with Affymetrix ExoSAP-IT
- Bidirectional di-deoxy sequencing was performed on an ABI 3730xl instrument
- Evolutionary history inferred by the Maximum Parsimony method was conducted with MEGA5 software

Results

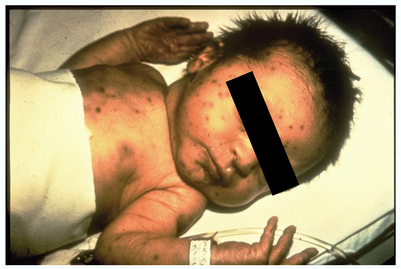

Figure 1: Infant with CRS displaying classic blueberry muffin purpura.

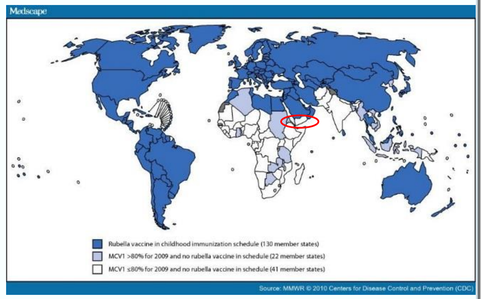

Figure 2: Global rubella immunization and MCV1 coverage rates.
Note: No RuV schedule in Yemen highlighted.

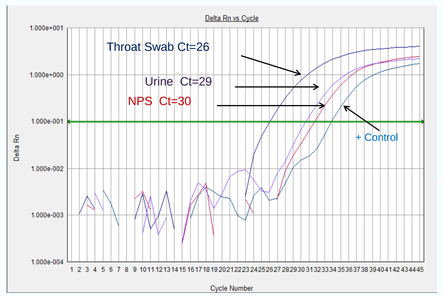

Figure 3: Real-time RT-PCR of viral nucleic acid from NPS, throat and urine samples with Cts of 30, 26 and 29 respectively.

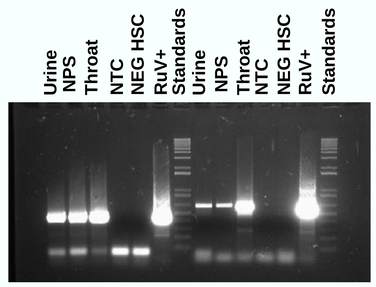

Figure 4. 1.0% TAE agarose gel of conventional rubella RT-PCR reaction products. Both PCR products were purified with Affymetrix Exo-SAP-IT and sequenced with the PCR primers.

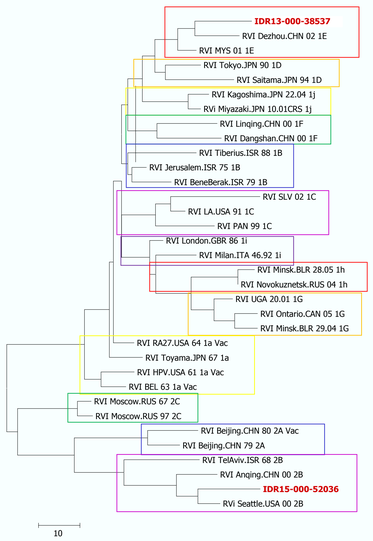

Figure 5: Phylogenetic analysis of the rubella E1 gene sequenced from the conventional RT-PCR products from samples from the two CRS cases.

A.

Collection Date	Sample Type	Ct
12/12/2013	Urine	29
	NPS	30
	Throat swab	26
1/14/2014	Throat Swab	31
	NPS	32
3/10/2014	NPS	25
5/9/2014	NPS	29
7/8/2014	NPS	Not Detected
10/21/2014	NPS	Not Detected
	OPS	Not Detected

B.

Collection Date	Sample Type	Ct
9/1/2015	Urine	36
	NPS	28
10/5/2015	Urine	36
	NP Wash	Not Detected
11/6/2015	NPS	Not Detected
11/17/2015	Urine	Not Detected
12/17/2015	Urine	Not Detected
	NPS	Not Detected

Figure 6: Rubella real-time RT-PCR of samples collected over several time points for patient 1(A) and 2(B).

Results

- Symptoms of CRS include cataracts, hearing impairment, congenital heart defects, microcephaly and purpura also known as blueberry muffin spots (Figure 1). Both infants had several symptoms consistent with CRS.
- Currently in Yemen the vaccination rate for MCV1 and Rubella remains below 75% and the leading cause of death in infants is due to vaccine preventable diseases (Figure 2).
- Real-time RT-PCR was used to detect Rubella virus (RuV) RNA in several sample types from two newborns whose mother's had both travelled to Yemen (Figure 3).
- Two conventional RT-PCR assays were used to generate a 945 bp fragment of the rubella E1 gene fragment for sequencing, from samples collected from both infants (Figure 4).
- Phylogenetic analysis of the rubella sequences from the two infants demonstrated that the viruses were distinct genotypes 1E and 2B, both known to be currently circulating in Yemen (Figure 5).
- Infants with CRS exhibit prolonged shedding of RuV as was seen with one of these cases (Figure 6).

Conclusions

- CRS is a severe and debilitating disease, preventable with prior vaccination of the mother
- Rubella virus was identified in several sample types from two infants whose mother's were unvaccinated and had both travelled to Yemen
- Phylogenetic analysis of a large portion of the rubella E1 gene determined the genotypes to be 1E and 2B, both of which are known to be currently circulating in Yemen
- Rubella virus shedding by CRS infants should be monitored after delivery as it can persist for several months
- The addition of these molecular methods into the Wadsworth Center Virology Laboratory, as part of the VPD Reference Lab services, proved invaluable in the diagnosis of CRS and these epidemiological investigations
- Non vaccinated individuals should be advised of the risks of travel to rubella endemic regions of the world particularly if there is a likelihood of pregnancy

Acknowledgements

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References Cited

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