Laboratory Investigations During Nipah Virus Outbreak in Kerala

work done and gaps identified

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Alert by physician in a private health care facility in Kozhikkode to virologist at MCVR, Manipal (distance ~300Km)

- May 17, 2018 – 1pm
- A 28 year old male admitted in early hours of 17 May – Encephalitis – Mechanical Ventilation – fall in saturation-Deteriorating rapidly
- His younger brother had died of similar illness 12 days back (May 5, 2018)
- His father, and an aunt also admitted with fever, bodyache and vomiting (Denies respiratory symptoms on repeated questioning) – No Hepatic and Renal involvement. No bleeding. His fiancée is admitted with mild fever
- May 17, 2018 – 7pm - Father, and aunt – Encephalitis – shifted to ICU

Directions by Virologist / Laboratory
- Collect, blood, Urine, Oropharyngeal swab in VTM or Endotracheal aspirate and CSF – Transportation by hand under cold chain.

Alert by State Surveillance officer and District Medical officer /District Surveillance officer - May 17, 2018 – 2-3pm

Receipt of samples at Laboratory
- May 18, 2017 – 8am Samples of four cases received (CSF from three cases)
Laboratory work up at MCVR, Manipal

- **May 18, 2018 – 8.30am** – Sample registration and aliquotting (BSL-2+)
  - **Clustering of Encephalitis** - Decides to undertake a comprehensive work up – parallel testing
  - Work up for Viral Encephalitis (HSV, JEV, VZV, Enterovirus, Mumps)
  - Additional tests for clustering of Encephalitis (Nipah and Chandipura Virus)
  - Acute Febrile illness (Leptospirosis, Scrub Typhus, Dengue, KFD, etc.)
  - Respiratory viruses (Influenza A/B, Adenovirus, MERS-CoV, Corona Viruses etc.)

- **May 18, 2018 – 5.30pm**
  - Releases report with negative result (except Nipah)
  - Three cases test positive for Nipah virus RNA (Blood, OP swab/ETA, Urine and CSF)
  - Re testing of Nipah with two additional protocols ordered
  - Physician informs death of first case – (He had already referred for autopsy - directed to collect samples)
Laboratory work up at MCVR, Manipal

• May 18, 2018 – 8.30pm
  • Nipah Virus confirmed in three patients. Fiancée test negative.
  • Alerts, Secretary DHR and DG ICMR and Director NIV, Pune by phone
  • Samples to be sent to NIV Pune for re-confirmation on May 19, 2018
  • State / district health and treating physician alerted –
    • Dangerous Virus
    • High Human to Human transmission
    • Name of the virus – Pending NIV re-confirmation

• May 19, 2018 – 8.00am – Samples dispatched to NIV Pune by hand

• May 19, 2018 -9.00pm – MCVR team reached Kozhikkode and joined State / district team – Coordination of sample transportation

• May 20, 2018 – 7.45pm – NIV re confirms the result

• May 20, 2018 – 8.00pM – Nipah Virus outbreak officially declared by DHS, Kerala State,
The following samples were collected from all suspected cases:

- **Oropharyngeal swabs**, placed in Viral Transport Medium (VTM).
  (If the patient was intubated, an endotracheal aspirate was collected)
- **Venous blood** (4ml) in vacutainer, (2ml) in EDTA
- **Sterile urine** (5ml) in sterile screw capped containers
- **CSF** (1-2ml) in encephalitis cases

Enhanced surveillance (Encephalitis and ARDS) – Contact tracing

All specimens collected by the HCWs and handed over to MCVR team

All samples were transported to MCVR under cold chain – Used railway for transportation – A system established and daily used since Aug 2009 (Pandemic Influenza time)

Samples were aliquoted under BSL- 3 conditions.

One aliquot of each specimen was inactivated with viral lysis buffer (Buffer AVL, Mat No. 1014777 QIAGEN, Germany); the other aliquots were stored at -80°C.
Nipah Virus diagnostic assays

• First line of testing (For diagnostic purpose)
  • Reverse Transcriptase Real Time PCR f(rRT-PCR) or NiV RNA:
    • Target: Nucleocapsid (N) gene
    • CDC recommended protocol - Lo M K et al (2012)

• Second line of testing (For research purpose only)
  • Serology: Serum samples were tested for anti-NiV IgM and IgG antibodies by CDC NiPah Virus IgM and IgG ELISA.

• Additional testing for other causes of Encephalitis / ARDS / AFI

### Results of Nipah Virus surveillance (n=360)
(17 May to 30 June 2018)

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>N (%)</th>
<th>Mortality(N)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nipah Virus</strong></td>
<td>18(5)</td>
<td>16</td>
</tr>
<tr>
<td>Influenzavirus B</td>
<td>20(5.5)</td>
<td>NA</td>
</tr>
<tr>
<td>Dengue Virus</td>
<td>19 (5.3)</td>
<td>1</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>10(2.8)</td>
<td>NA</td>
</tr>
<tr>
<td>Shigella spp.</td>
<td>3(0.8)</td>
<td>NA</td>
</tr>
<tr>
<td>West Nile Virus</td>
<td>2(0.6)</td>
<td>NA</td>
</tr>
<tr>
<td>Japanese Encephalitis Virus</td>
<td>1(0.3)</td>
<td>1</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>1(0.3)</td>
<td>NA</td>
</tr>
<tr>
<td>HSV-1</td>
<td>1(0.3)</td>
<td>NA</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>1(0.3)</td>
<td>NA</td>
</tr>
<tr>
<td>Rota virus</td>
<td>1(0.3)</td>
<td>NA</td>
</tr>
</tbody>
</table>
Comparison of Nipah Virus Real Time PCR protocols (Malaysian vs CDC) (n=30)

<table>
<thead>
<tr>
<th>Malaysian Protocol</th>
<th>CDC Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
</tr>
</tbody>
</table>

Malaysian protocol - Guillaume, V et.al., 2004..
CDC Protocol - Lo, M.K et.al. 2012..

Unpublished data
## Nipah Virus Serology results (n=17)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-NiV IgM only positive in acute sample</td>
<td>8</td>
</tr>
<tr>
<td>Anti- NiV IgM and IgG positive in acute sample</td>
<td>4</td>
</tr>
<tr>
<td>Anti- NiV IgM and IgG Negative in acute sample</td>
<td>5</td>
</tr>
</tbody>
</table>
**NiV – Kerala outbreak - Phylogenetic Analysis**

- The NiV isolated from the Kerala outbreak grouped with genotype B viruses but formed a diversified subclade.
- Sequence analysis of the current outbreak NiV revealed 97.23% similarity to the Bangladesh lineage.
Percentage positivity and relative load of NiV virus RNA in different clinical specimens during acute and convalescent period

Unpublished data
Persistence of RNA in semen
Cellular immune response in NiV disease
Studies based on two survivors

Unpublished data
Cellular immune response in NiV disease
Studies based on two survivors

Unpublished data
Cellular immune response in NiV disease
Studies based on two survivors

Unpublished data
Antibody response in NiV and RNA level
Studies based on two survivors

Unpublished data
NiV POC rRT-PCT test development

- True\text{nat} Test Chips
- True\text{lab} Uno Dx

LTCC Micro Thermal Cycler

Smart chip (flash):
- Test identifier
- Standard curve
- Lot information
- Expiry date, etc.
NiV POC rRT-PCT test development

Truenat™ chip based Point of Care Real Time micro PCR - PCR – an indigenously developed assay for the detection of NiV virus in clinical samples

Truenat™ Result: Detailed view

Truenat™ Result: Optical view

Truenat™ micro PCR chips for Nipah virus assay
### NiV POC rRT-PCT test development

#### Table 1: Comparison of CT values

<table>
<thead>
<tr>
<th>Sample No</th>
<th>CDC protocol-CT values</th>
<th>Truenat™ microchip PCR-CT values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31</td>
<td>30.8</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>31.6</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>23</td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>33.08</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>26.8</td>
</tr>
<tr>
<td>7</td>
<td>28</td>
<td>28</td>
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<td>8</td>
<td>30</td>
<td>31.67</td>
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<td>9</td>
<td>22</td>
<td>24.5</td>
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<tr>
<td>10</td>
<td>34</td>
<td>34.6</td>
</tr>
<tr>
<td>11</td>
<td>NEGATIVE</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>NEGATIVE</td>
<td>ND</td>
</tr>
<tr>
<td>13</td>
<td>NEGATIVE</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td>NEGATIVE</td>
<td>ND</td>
</tr>
<tr>
<td>15</td>
<td>NEGATIVE</td>
<td>ND</td>
</tr>
<tr>
<td>16</td>
<td>NEGATIVE</td>
<td>ND</td>
</tr>
<tr>
<td>17</td>
<td>NEGATIVE</td>
<td>ND</td>
</tr>
<tr>
<td>18</td>
<td>NEGATIVE</td>
<td>ND</td>
</tr>
<tr>
<td>19</td>
<td>NEGATIVE</td>
<td>ND</td>
</tr>
<tr>
<td>20</td>
<td>NEGATIVE</td>
<td>ND</td>
</tr>
<tr>
<td>21</td>
<td>NEGATIVE</td>
<td>ND</td>
</tr>
<tr>
<td>22</td>
<td>NEGATIVE</td>
<td>ND</td>
</tr>
</tbody>
</table>

#### Table 2: Performance of Truenat™ microchip PCR with reference to the CDC protocol

<table>
<thead>
<tr>
<th>Truenat NiV virus</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>9</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90.00</td>
<td>100.00</td>
<td>100.00</td>
<td>92.31</td>
</tr>
</tbody>
</table>

Unpublished data
Testing of environmental samples

- 60 environmental samples including partially eaten mangoes, guava and areca nuts with bite marks of bats were collected from the surroundings of the residence and potential work places of the index case.
- None had evidence of NiV RNA by real time PCR.
• As per directions of the DHR/ICMR, MoHFW, GoI, all NiV positive specimens stored at MCVR were handed over to the National Institute of Virology (NIV), Pune on 11th July 2018.
Gaps identified

- Challenges in sample collection – Who will collect?
- Case definition – No adherence – Asymptomatic cases being samples – No NEGATIVE predictive value
- Unavailability of POC tests – Positive and Negative cases simultaneously in isolation ward
- Suspicion of NiV hampered other diagnosis
- Inadequate surveillance/ testing of encephalitis cases
- Routine AES surveillance is not enough for detecting NiV
  - Need to change approach
  - CSF may not be the best specimen
  - Oropharyngeal swab is the preferred specimen
  - ILI/ SARI cases also should be tested for NiV
- Unavailability of antibody based tests for documenting asymptomatic cases
• **Key to early detection of NiV and other emerging diseases**
  
  • Good laboratory supported surveillance
  • Good communication between hospital, health services and laboratory
  • Diagnostic facility to identify common diseases at Taluka and District level hospitals
  • Encourage referrals of specimens and networking of laboratories
  • Preparedness of the laboratory including provision for handling the load during an outbreak
Thank you

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