USING ADVANCED GENOMIC ANALYSIS FOR POTENTIAL HAEMORRHAGIC FEVER CAUSATIVE AGENTS

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Viral Hemorrhagic Fevers Study in Ghana

• Pertinent questions before initiation......

  – Some known VHFs were endemic in parts of West Africa, however conditions not found in neighboring countries although animal vectors for these VHFs were distributed throughout the region

  – Global estimates were flawed because surveillance for cases of such diseases were not uniformly performed
Viral Haemorrhagic Fevers (VHFs)

• A group of febrile highly infectious illnesses and often fatal diseases caused by several viral families

• Four distinct families of RNA viruses:
  • Arenaviridae: Lassa fever virus and a group of viruses referred to as the New World arenaviruses
  • Bunyaviridae: Crimean Congo HF virus, Rift Valley fever virus, and agents of haemorrhagic fever with renal syndrome
  • Filoviridae: Ebola and Marburg viruses
  • Flaviviridae: Dengue, Yellow fever and others
What was Happening

- Reports of importation of cases into non-endemic regions
  - AV strain of Lassa
  - Yellow fever
  - Dengue fever etc

- High numbers of suspected cases of viral haemorrhagic fevers (Yellow Fever) in Northern Ghana

<table>
<thead>
<tr>
<th>REGION</th>
<th>Suspected Cases</th>
<th>Confirmed (Death)</th>
<th>Suspected Cases</th>
<th>Confirmed (Death)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brong Ahafo</td>
<td>37</td>
<td>5(2)</td>
<td>15</td>
<td>2(0)</td>
</tr>
<tr>
<td>Northern</td>
<td>86</td>
<td>7(0)</td>
<td>14</td>
<td>1(0)</td>
</tr>
<tr>
<td>Upper West</td>
<td>97</td>
<td>9(2)</td>
<td>12</td>
<td>4(1)</td>
</tr>
<tr>
<td>Upper East</td>
<td>73</td>
<td>2(0)</td>
<td>31</td>
<td>6(2)</td>
</tr>
<tr>
<td>Total</td>
<td>293</td>
<td>23(4)</td>
<td>72</td>
<td>13(3)</td>
</tr>
</tbody>
</table>

Courtesy: Disease Surveillance Department, Ghana Health Service

Problem

• Suspected Viral Haemorrhagic Fever cases were only tested for Yellow Fever and not other known viruses that share similar symptoms.

• However, of the unconfirmed cases, fatalities were recorded.

• It was therefore necessary to extend laboratory investigations into other haemorrhagic fever and other potential viruses mimicking symptoms.
Rationale

• To determine the viral agents responsible for the haemorrhagic fever symptoms

• To provide useful information to enable public health authorities design appropriate control and intervention measures

• To expand the research infrastructure that will facilitate haemorrhagic fever surveillance in Ghana
Study sites and Why?

• Previous reports of Haemorrhagic Fever viruses

• Share borders with countries with history of HF viruses

• Game reserve (Mole National Park) – potential of zoonotic infections
• In total, 300+ clinical specimens of serum/plasma were collected

• Molecular tools were used for a panel including,
  • Ebola/Marburg, Lassa, Yellow fever, Dengue fever, Rift Valley and Chikungunya

• Differential diagnosis – Viral Hepatitis
• The study indicated a possible circulation of Lassa, Dengue 2 and Chikungunya

• It also suggested that viral hepatitis infections, which often share clinical symptoms with VHFs were widespread.

• Established an algorithm for lab investigations of known VHFs
  • To expand the research infrastructure that will facilitate haemorrhagic fever surveillance in Ghana

• Provided useful information to public health authorities for appropriate control and intervention measures
Hospital-Based Surveillance for Viral Hemorrhagic Fevers and Hepatitides in Ghana

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The incubation period is 6 to 21 days. The onset of LF illness is gradual, with non-specific signs and symptoms starting with fever, general weakness and malaise. After a few days, headache, sore throat, muscle pain, back pain, vomiting, diarrhoea and abdominal pain may follow. Severe cases may progress to show facial swelling, bleeding from mouth, nose, vagina or gastrointestinal tract, and low blood pressure.

Imported Lassa fever: a report of 2 cases in Ghana

Nicholas N.A. Kyei1, Mark M. Abiba1, Foster K. Kwawu1, Prince G. Agbenohevi1, Joseph H.K. Bonney1, Thomas K. Agbekempe1, Shirley C. Nimo-Paintsil1, William Ampofo1, Sally-Ann Ohene2 and Edward O. Nyarko1

Fatal hepatitis E viral infection in pregnant women in Ghana: a case series

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2014 EVD Outbreak

- Largest Ebola epidemic in history - affected multiple countries including Guinea, Liberia, Sierra Leone
- A recorded case fatality rate of 53%

### Countries with Widespread Transmission

<table>
<thead>
<tr>
<th>Country</th>
<th>Start of Outbreak (2014)</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea</td>
<td>February</td>
<td>3,814</td>
<td>2,544</td>
</tr>
<tr>
<td>Liberia</td>
<td>March</td>
<td>10,678</td>
<td>4,810</td>
</tr>
<tr>
<td>Sierra Leone</td>
<td>May</td>
<td>14,124</td>
<td>3,956</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>28,616</td>
<td>15,227</td>
</tr>
</tbody>
</table>

### Countries Previously Affected

<table>
<thead>
<tr>
<th>Country</th>
<th>Case(s)</th>
<th>Death(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nigeria</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>USA</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Senegal</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Mali</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Spain</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>UK</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Italy</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
In-Country Activities

- Emergency response activities were activated
  - Establishment of a national EVD preparedness and response plan to set up a surveillance system for detection
  - Designation of NMIMR as the main lab for investigations
- All suspected cases were submitted for testing
## Total Number Received and Tested

<table>
<thead>
<tr>
<th>Year</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>134</td>
<td>27</td>
<td>43</td>
</tr>
</tbody>
</table>

- Total number as of December 2016 is **204**
- Six of which were received from Togo
The question then is ..... 
What could possibly be the etiology for these cases if initial suspicions could not be confirmed

- So the deployment of NGS techniques to find answers.....
Why Cambridge

• Embark on a 6-month fellowship under the CAPREx initiative

• Work directly with Simon Frost on a research titled, “Using advanced genomic analysis for potential hemorrhagic fever causative agents”

• Objective:
  • To identify the infectious agent(s) in residual clinical specimens that have tested negative by RT-PCR for Ebola and other haemorrhagic fever viruses using next generation sequencing techniques
Sample Preparation

Nucleic Acid extraction and Purification
• Specimen manipulations in the BSL 3 laboratory
• TRIzol reagent – effectively inhibits RNase activity

SISPA PCRs and Fragments Precipitation
• A random priming method that allows enrichment of viral genomes in only few steps
• Ethanol precipitation to concentrate the nucleic acid

Ribosomal RNA Depletion / Removal
• Removal of rRNA from the samples
• Wash and resuspend magnetic beads which binds to removal probes hybridized to rRNA

Quantification and Assessment of Nucleic Acid
• Use Qubit Fluorometric Quantitation to quantitate and assess the integrity and quality of nucleic acid
• Use Agilent 2100 Bioanalyzer to size and quality control the nucleic acid
In conclusion

• Good success and great opportunity for career progression

Whilst there.......  

• Attended 2 Scientific Meetings
  • The International Conference on (Re-)emerging Infectious Diseases, Addis Ababa, Ethiopia
  • Virus Genomics and Evolution 2018 Conference, Hinxton UK
• DARPA grant won to work to work on Bats and Henipaviruses
  • Lead NMIMR Work package
Appreciation
• CAPREx Secretariat

Professors,
• Simon Frost
• James Wood
• Jon Heeney
• William Ampofo
• Kwadwo Koram
• Alex Nyarko

Thanks for your attention!

Q & A !!