Understanding all Steps of Nipah Virus Transmission

Steve Luby, MD

Zoonoses and emergence of new infectious diseases: biology meets anthropology
10 June 2013
Collège de France, Paris
Malaysia Nipah Outbreak

- September 1998 – May 1999
- 283 human cases of acute encephalitis
  - 109 deaths
  - Case fatality rate 39%
- Paul Chua isolated a novel paramyxovirus from a patient in Kampung Sungai Nipah village
Nipah Pathology

- Causes a diffuse vasculitis
- The brain is the most severely affected organ – tropism to the brainstem
- Virus commonly identified in
  - lung
  - kidney

Guinea Pig Brain with NiV
From Corrie Brown, UGA
How did people contract Nipah Virus in Malaysia?

• The outbreak was concentrated among pig farmers
  – 92% of cases reported contact with pigs

• Compared to controls, persons with Nipah encephalitis were
  – 5.6 times more likely to have close contact with pigs.
  – 3.7 times more likely to have contact with sick pigs
Singapore Outbreak

March 1999

- Outbreak among 11 abattoir workers in Singapore
  - 1 death
  - All worked processing pigs imported from Malaysia

From where did the pigs get Nipah?
Nipah wild animal studies

• Numerous wild animals trapped and tested
• 8 different species of fruit bats sampled
  – 4 of the 8 species had antibodies against Nipah virus.
• Nipah virus isolated
  – Urine from *Pteropus hypomelanus* in Malaysia
Index farm

- 30,000+ pigs
- Adjacent to primary forest, fruit bat habitat
- Network of other large farms close by

Slide courtesy of Peter Daszak
Why did Nipah virus emerge in 1998?

Malaysia Outbreak Control

• Outbreak ceased following the culling of over 900,000 pigs
  – Fruit trees no longer permitted above pig pens
  – Pork industry decimated

• No subsequent cases of Nipah recognized in Malaysia from people or animals

Photo: BBC News
2001
Siliguri
Meherpur
66 cases 49 deaths
Meherpur
13 cases 9 deaths
2002
No cases
2003
Naogaon
12 cases 8 deaths
2004
Rajbari
31 cases 23 deaths
Faridpur
36 cases 27 deaths
2005
Tangail
12 cases 11 deaths
2006
No cases
2007
Thakurgaon
7 cases 3 deaths
Kushtia
8 cases 5 deaths
Nadia
5 cases 5 deaths
2008
Manikgonj
4 cases 4 deaths
Rajbari
6 cases 5 deaths
2009
Rangpur, Gaibandha, Rajbari, Nipharamari
4 cases 1 death
2010
Faridpur, Rajbari, Gopalgonj, Kurigram
17 cases 15 deaths
2011
Lalmonirhat, + 5 other districts
28 cases 28 deaths
2012
Joypurhat, Rajshahi
13 cases 10 deaths
2013
13 districts
24 cases 21 deaths
Total
290 cases 225 deaths
Case fatality : 78%
**Pteropus giganteus in Bangladesh**

<table>
<thead>
<tr>
<th>Year</th>
<th>Bats Tested</th>
<th>Nipah IgG+</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>92</td>
<td>48</td>
<td>52%</td>
</tr>
<tr>
<td>2006</td>
<td>81</td>
<td>15</td>
<td>19%</td>
</tr>
<tr>
<td>2007</td>
<td>218</td>
<td>107</td>
<td>49%</td>
</tr>
</tbody>
</table>

How does Nipah virus transmit from wildlife to humans in Bangladesh?
11 January 2005

- Government health workers reported that 8 previously healthy persons from Basail Upazila died within a one week period.
Case definition: Fever +
  – Mental status changes
  – Seizures:

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Count (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>12 (100%)</td>
</tr>
<tr>
<td>Seizures</td>
<td>4 (33%)</td>
</tr>
<tr>
<td>Unconsciousness</td>
<td>9 (75%)</td>
</tr>
<tr>
<td>Headache</td>
<td>5 (42%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>5 (42%)</td>
</tr>
<tr>
<td>Difficulty breathing</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>Death</td>
<td>11 (92%)</td>
</tr>
</tbody>
</table>

- Median time from first symptom to death: 4 days
Onset of illness
Tangail District, Bangladesh, 2005 (N=12)

Number

illness onset

Dead
Survived

2-Jan
4-Jan
6-Jan
8-Jan
10-Jan
12-Jan
14-Jan
16-Jan
Case location Tangail Nipah Outbreak 2005
Risk factor study

- Design: Case Control
- Cases: All 12 persons meeting the case definition enrolled
- 3 controls per case
  - Next closest house, person nearest in age
  - Proxies for persons who had died
## Case Control Results

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>No. and % of cases with this risk factor</th>
<th>No. and % of controls with this risk factor</th>
<th>Odds Ratio</th>
<th>95% confidence limit</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical contact with sick animal</td>
<td>5 (42)</td>
<td>5 (14)</td>
<td>4.4</td>
<td>0.9,20.4</td>
<td>0.09</td>
</tr>
<tr>
<td>Physical contact with sick chicken</td>
<td>3(25)</td>
<td>3(8)</td>
<td>3.7</td>
<td>0.5,24</td>
<td>0.16</td>
</tr>
<tr>
<td>Killed a sick animal</td>
<td>1(8)</td>
<td>2(6)</td>
<td>1.6</td>
<td>0.05,22</td>
<td>1.00</td>
</tr>
<tr>
<td>Ate any sick animal</td>
<td>1(8)</td>
<td>2(6)</td>
<td>1.6</td>
<td>0.05,22</td>
<td>1.00</td>
</tr>
<tr>
<td>Seen fruit bats during daytime</td>
<td>3(25)</td>
<td>5(14)</td>
<td>2.1</td>
<td>0.34,11</td>
<td>0.39</td>
</tr>
<tr>
<td>Seen fruit bats during nighttime</td>
<td>8(67)</td>
<td>13(36)</td>
<td>3.5</td>
<td>0.87,15.4</td>
<td>0.06</td>
</tr>
<tr>
<td>Drank raw date palm sap</td>
<td>7(58)</td>
<td>6(17)</td>
<td>7.0</td>
<td>1.6,31</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Date Palm Sap Collection

• Late November through March
  – Sap harvesters cut a tap is cut into the tree
    • In the evening they place a clay pot under the tap
    • Each morning the pot is removed
  – Most sap is made into molasses
  – Some sold fresh early in the morning
    • A local delicacy
Date Palm Sap Distribution
Habla Union

• One of the fatal cases was the son of a date palm sap collector
  – drank date palm sap daily

• Heard bats in his date palm trees at night
  – Found bat excrement on his pots

• Several days prior to the outbreak he sent date palm sap to his relatives in a nearby homestead.
  – 3 cases occurred in the family
## Date palm sap transmission of NIV

### Epidemiological Evidence

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Cases Exposed (%)</th>
<th>Controls Exposed (%)</th>
<th>Odds Ratio</th>
<th>95% Confidence Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>Tangail</td>
<td>58</td>
<td>17</td>
<td>7.0</td>
<td>1.6, 31</td>
</tr>
<tr>
<td>2008</td>
<td>Manikgonj</td>
<td>100</td>
<td>25</td>
<td>18</td>
<td>2.2, inf</td>
</tr>
<tr>
<td>2010</td>
<td>Faridpur</td>
<td>69</td>
<td>30</td>
<td>5.2</td>
<td>1.2, 26</td>
</tr>
<tr>
<td>2011</td>
<td>Lalmonirhat</td>
<td>68</td>
<td>11</td>
<td>17</td>
<td>4.0, 70</td>
</tr>
</tbody>
</table>
We knew

- Pteropus bats occasionally shed Nipah virus RNA in their saliva
  - Reynes et al, Emerg Infect Dis 11: 1042-7

- Date palm sap implicated in outbreak investigations
- Any sap we collected was well after the outbreak

Henipavirus survival in fruit juice at 22 °C.

Infrared wildlife photography

Salah Uddin Khan

Manikgonj Outbreak 2008

- 7 trees where implicated date palm sap was collected
- 7 nights of observation
- Mean 15 bat visits per night
- Bats licked the sap mean 8.4 times per night
- 49% of bats were *Pteropus*

Domestic Animal Nipah Transmission in Bangladesh

• 2001 Meherpur
  – Contact with a sick cow
  – Odds ratio 6.9 (2.2, 27.7)

• 2003 Naogaon
  – Close proximity to a pig herd
  – Odds ratio 6.1 (1.4, 25.9)

• 2004 Rajbari
  – 2 goats developed fever, difficulty walking, and died
  – 2 weeks later a child who frequently played with goats developed Nipah encephalitis

Pig herd in Bangladesh
Photo: Salah Uddin Khan
Henipah Virus infections in cattle and goats?

- Veterinary field team visited sites of 5 previous human outbreaks of Nipah virus
  - Located the bat roost closest to the highest concentration of human cases
  - Within 1000 meter radius
    - 80 cattle (400 total)
    - 80 goats (400 total)
- Administered questionnaire on exposures
- Samples sent to Australian Animal Health Laboratory for testing
  - Luminex antibody
    - against G and F protein
    - Nipah and Hendra
  - Viral neutralization
### Domestic Animal Henipavirus

<table>
<thead>
<tr>
<th></th>
<th>No. (%) Luminex sero-positive</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nipah</td>
<td>Hendra</td>
</tr>
<tr>
<td><strong>Cattle (n=400)</strong></td>
<td>11 (2.8)</td>
<td>3 (0.8)</td>
</tr>
<tr>
<td><strong>Goat (n=400)</strong></td>
<td>9 (2.3)</td>
<td>1 (0.3)</td>
</tr>
</tbody>
</table>

- **All Luminex positive sera negative for viral neutralization**
- **Cross reactivity with an unknown henipavirus?**

<table>
<thead>
<tr>
<th></th>
<th>Luminex henipavirus positive n (%)</th>
<th>Luminex henipavirus negative n (%)</th>
<th>odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fed off partially animal eaten fruits</strong></td>
<td>8 (57)</td>
<td>88 (23)</td>
<td>4.2 (1.5-11.9)</td>
</tr>
<tr>
<td><strong>Drank raw palm juice</strong></td>
<td>2 (14)</td>
<td>7 (2)</td>
<td>7.2 (1.9-27.7)</td>
</tr>
</tbody>
</table>
Novel paramyxovirus from *P. giganteus* bats in Bangladesh

Simon Anthony, Under review
How else is Nipah virus transmitted in Bangladesh?
Dates of illness onset from Faridpur outbreak coded by transmission generation (N=36)

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Dates of illness onset from Faridpur outbreak coded by transmission generation (N=36)

Faridpur Cohort Study

- Touching a Nipah patient who later died (RR 15.0, 95% CI 4.0, 65)
- Touching an unconscious patient (RR 4.5, 95% CI 1.7, 12)
- Touching a patient with respiratory symptoms (RR 5.0, 95% CI 2.0, 14)
- Washing hands after contact with Patient F (RR 0.20, 95% CI 0.03, 0.90)

Male, 40 yrs, taken on 6th day of illness, died 2 days later

Male, 35 yrs, taken on 5th day of illness, died next day
Partial NiV N-ORF Maximum Parsimony Bootstrap consensus tree 1000 replicates
(Proposed 729 nt NiV N gene genotyping window)

Slide: Michael Lo, CDC

Phenotypic diversity of Nipah Virus

- 8 ferrets
  - inoculated with NiV strain from Bangladesh

- 7 ferrets
  - inoculated with NiV strain from Malaysia

- Mean viral RNA levels in respiratory secretions
  - 10x higher among ferrets inoculated with the Bangladesh strain

Anthropological Investigation

• May 2004 to January 2005

• In-depth interviews with:
  – family members in households where a Nipah case occurred
  – neighboring families
  – local health practitioners
  – hospital workers
  – date palm sap collectors
  – bat catchers

Family caregivers during Faridpur outbreak

• Families provide direct care
  – Rooted in emotional support
  – Cultural expectation to
    • maintain close physical contact during illness
    • Provide hands-on care with direct contact with patient’s body fluids

• Desire for close physical contact before dying (hug to say goodbye, feed sick patient, whisper Koranic verses in ear)

• Family members and religious leaders prepare and cleanse the body, particularly the orifices, for burial

Date Palm Sap Harvesting Practices
Anthropological Investigation

- Harvesters typically tend 50 – 100 trees
  - Seasonal work
  - Share half of sap with owner
  - Earn median 5 US$ per week selling sap
- Bats are somewhat of a nuisance
- Occasional methods to discourage bats
  - Thorns
  - Bending leaves
  - Spreading lime
  - Bamboo nets

Photo: Nazmun Nahar
Bamboo nets

- Obstructs access to jar and shaved part of the tree
- Rarely used

Photo: Nazmun Nahar

Reducing date palm sap contamination by bats
a randomized controlled trial

• Selected 120 date palm sap producing trees in a village
• Randomly assigned four types of interventions to 15 trees each to cover the shaved part, sap stream, tap and collection pot:
  – bamboo skirt
  – dhoincha (local plant) skirt
  – jute stick skirt
  – polythene
• 60 trees enrolled as controls
• The controls were matched on:
  – apparent height
  – shaving pattern


Photo: Nazmun Nahar
Jute

Doincha

Bamboo

Polyethylene

Salah Uddin Khan
## Bat Visits

<table>
<thead>
<tr>
<th></th>
<th>Bamboo</th>
<th>Dhoincha</th>
<th>Jute</th>
<th>Poly ethylene</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bat visits on and around tree</td>
<td>176</td>
<td>45</td>
<td>125</td>
<td>112</td>
<td>4630</td>
</tr>
<tr>
<td>% landed on the tree</td>
<td>20</td>
<td>18</td>
<td>43</td>
<td>11</td>
<td>78</td>
</tr>
<tr>
<td>Number contacting date palm sap</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>3556</td>
</tr>
<tr>
<td>% contacting sap</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>76</td>
</tr>
</tbody>
</table>

Sap Harvester Acceptability Trial

- **Intervention:**
  - Community meetings targeting 79 tree owners and 79 *gacchis*

- **Baseline:** No bamboo skirts used in the community

- **One month after intervention**
  - 34% of *gacchis* used skirts
  - 14% of tree owners used skirts

Rebeca Sultana

Photo: Jon Epstein
District Level Prevention Trial
2013

• Objective:
  – prevent human consumption of raw sap

• Site
  – Intervention in 348 villages in Rajbari District
  – Control Kushtia district

• Behavior change communication intervention
  – Posters, video documentary, 45 second television spot
  – Trained local non-government organization health communicators
কাঁচা রুস (খেতে) ধাত
বাতায় ঢাকা রুস-ই খাত
<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
<th>Images</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wash your hands with soap</td>
<td><img src="image1.png" alt="Image 1" /> <img src="image2.png" alt="Image 2" /></td>
</tr>
<tr>
<td></td>
<td>Before you eat</td>
<td><img src="image3.png" alt="Image 3" /></td>
</tr>
<tr>
<td></td>
<td>After you feed the patient</td>
<td><img src="image4.png" alt="Image 4" /></td>
</tr>
<tr>
<td></td>
<td>After you clean the patient</td>
<td><img src="image5.png" alt="Image 5" /></td>
</tr>
<tr>
<td>2</td>
<td>Keep your food and the patient's food in separate bowls or plates</td>
<td><img src="image6.png" alt="Image 6" /></td>
</tr>
<tr>
<td></td>
<td>Store the patient's food when they are finished</td>
<td><img src="image7.png" alt="Image 7" /></td>
</tr>
<tr>
<td></td>
<td>Eat the patient's leftovers <strong>DO NOT</strong></td>
<td><img src="image8.png" alt="Image 8" /></td>
</tr>
</tbody>
</table>
3. Try to sleep in a separate bed or on the floor. Otherwise, sleep with your back to the patient. Sleep face to face with the patient.

4. When you hug and comfort your patient, put your head to their chest. Or, put their head to your chest. Keep your face less than 1 hand’s distance from your patient’s face.
Hospital Handwashing Pilot

- **Objective:**
  - Pilot 2 low cost hand hygiene stations
  - Assess impact on staff and attendant handwashing

- **Site**
  - Gazipur District Hospital
  - Fardipur Medical College Hospital

- **Behavior change communication intervention**

- **Measurements**
  - Baseline observation
  - Process evaluation
  - Endline observation
  - In-depth interview
Nipah virus

- Recurrent outbreaks
- High case fatality
- Person to person transmission
- Global risk

Requires

- Global support
- Multi-disciplinary engagement
  - Government health authorities, physicians, virologists, veterinarians, anthropologists, wildlife ecologists, behavior change experts
- Multi-sector approach

Gross Domestic Product

Trillion US$
Acknowledgements

• Government of Bangladesh
  – Institute for Epidemiology Disease Control and Research (IEDCR)
    • Mahmudur Rahman, Be-Nazir Ahmed, Mustak Hossein
  – Civil surgeons
  – Government Hospitals
  – Department of Forestry

• ICDDR,B

• Centers for Disease Control and Prevention
  – Pierre E. Rollin, James A. Comer, Paul Rota, Michael Lo, Stewart Nichols, James Sejvar, Rob Breiman, Joel Montgomery

• EcoHealth Alliance
  • Jon Epstein, Peter Daszak, Simon Anthony

• FHI360
  – Susan Zimicki, Fernando Garcia

• Funding
  – Government of Bangladesh
  – Centers for Disease Control and Prevention
  – National Institutes of Health, Fogarty International Center
  – USAID
  – National Institutes of Health, IH, DMID, ICIDR