Why are so few people getting infected in face of large exposed population?

Peter Horby
Oxford University Clinical Research Unit
Vietnam
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Is premise correct?
Are few people getting infected in the face of large numbers exposed?
Poultry and human cases

Human cases under-recognized?

A patient with suspected avian flu in Asia
### Unconfirmed cases in clusters

7/34 = 20%

#### Kamphaeng Phet, THA
- 11y Daughter, Onset: 2/Sep 04
- 26y Mother, Onset: 11/Sep 04
- 32y Aunt, Onset: 16/Sep 04

#### Ha Nam, VTN
- 12y Daughter, Onset: 25/Dec 03
- 30y Mother, Onset: 01/Jan 04

#### Thai Binh, VTN
- 31y Brother, Onset: 03/Jan 04
- 30y Sister, Onset: 10/Jan 04
- 23y Sister, Onset: 10/Jan 04

#### Nam Dinh, VTN
- 7y Sister, Onset: 29/Dec 03
- 5y Brother, Onset: 01/Jan 04

#### Hau Giang, VTN
- 19y Brother, Onset: 27/Jul 04
- 25y Sister, Onset: 05/Aug 04

#### Kampot, CAM
- 25y Sister, Onset: 26/Jan 05
- 14y Brother, Onset: 20/Jan 05

#### Dong Thap, VTN
- 46y Brother, Onset: 5/Jan 05
- 42y Brother, Onset: 10/Jan 05
- 36y Brother, Asymptomatic
- 21y Brother, Onset: 14/Feb 05
- 81y Grandfather
- 26y M nurse, Onset: 24/Feb 05
- 69y Husband, Onset: 19/Feb 05
- 61y Wife, Asymptomatic

#### Sukhothai, THA
- 6y Son, Onset: unknown
- 6y Son, Onset: same as above

#### Hai Phong, VTN
- 35y Father
- 33y Mother
- 13y Daughter
- 10y Daughter
- 4mo Daughter

#### Quang Binh, VTN
- 13y Sister, died on 9/Mar 05
- 5y Brother, Admit on 15/Mar, Onset?

### Sub-clinical / mild cases

Hong Kong = 5; Vietnam=3, Turkey=1, Indonesia=3+

<table>
<thead>
<tr>
<th>Group</th>
<th>Location</th>
<th>Year</th>
<th>Assay</th>
<th>No.</th>
<th>No. +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry workers</td>
<td>HK</td>
<td>1997</td>
<td>MN</td>
<td>1525</td>
<td>10%</td>
</tr>
<tr>
<td>Poultry cutters</td>
<td>HK</td>
<td>1997</td>
<td>MN</td>
<td>293</td>
<td>9</td>
</tr>
<tr>
<td>HCW</td>
<td>HK</td>
<td>1997</td>
<td>MN</td>
<td>528</td>
<td>10</td>
</tr>
<tr>
<td>Household contacts</td>
<td>Viet Nam (S)</td>
<td>2004</td>
<td>MN</td>
<td>51</td>
<td>0</td>
</tr>
<tr>
<td>Contacts of sick poultry</td>
<td>Viet Nam (S)</td>
<td>2004</td>
<td>MN</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Household contacts</td>
<td>Vietnam (S)</td>
<td>2004</td>
<td>RT-PCR</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>HCW with contact</td>
<td>Viet Nam (S)</td>
<td>2004</td>
<td>ELISA, MN</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>HCW with contact</td>
<td>Viet Nam (N)</td>
<td>2003-4</td>
<td>MN, WB</td>
<td>83</td>
<td>0</td>
</tr>
<tr>
<td>HCW with contact</td>
<td>Thailand</td>
<td>2004</td>
<td>Clinical only</td>
<td>54</td>
<td>0</td>
</tr>
<tr>
<td>HCW with contact</td>
<td>Thailand</td>
<td>2004</td>
<td>MN (paired sera)</td>
<td>25</td>
<td>0</td>
</tr>
</tbody>
</table>
### Conclusion

1. There are more severe cases than reported
2. There are sub-clinical cases - but unlikely to be large numbers
3. Never the less, there are a small number of cases given the large number of people exposed
4. There is a substantial “barrier to transmission”
Gaps & actions

- Interpretation of serosurveys
- Estimate of true case incidence
- Estimate of numbers exposed
- Estimate of risk
- Study kinetics of antibody responses in survivors & controls
- Systematic review & meta-analysis with estimates of uncertainty
- Estimate of incidence + estimate of exposure = estimate risk

Why we need a ‘risk assessment’

- Risk reduction – honest education
- Advocacy
- Resource allocation
- Base-line for surveillance & monitoring
- Base-line for evaluation
- Identify risk groups (familial relative risk)
- Guide science (e.g. sample size)
Why so few cases?

What are barriers to transmission?
Virus?
Yes...but role of heterogeneity?

Variations
– binding, replication, immune stimulation

Dose / exposure intensity?

Behaviour
Chance
Biology
But.....

- Cullers exposed to high doses
- Many people exposed to ‘sufficient dose’
  - Birds excrete a lot of virus
  - Environmental contamination widespread
- 30% of cases do not report any poultry exposure
- 25% of controls report high risk exposure

Site of inoculation?

- Lower respiratory tract
- Water ingestion
- Water on conjunctiva
- Food ingestion
  (poultry products, contaminated vegetables)
But........

- Case-control studies have generally not indentified important ‘unusual exposures’
- Attributable risk of ‘unusual exposures’?
  - Swimming in rivers & lakes
  - Raw ducks blood
- Direct contact with sick birds is main risk
- A common risk:
  - Cullers
  - 25% of controls report high risk exposure
  - Population attributable risk percent is only 28%
- 30% of cases do not report any poultry exposure

The victim?

Host heterogeneity
Host factors

- Immunity? – antibodies or CMI
  - Age profile
  - Cullers
- Intrinsic susceptibility?
  - Binding
  - Replication
  - Immune response
  - Release
  - Genotypic
  - Phenotypic – co-factors (age, illness...)

Genetic susceptibility?

Some features of H5N1 epidemiology suggest a host genetic influence on susceptibility to infection:

- Family clustering of cases (*bias*?)
- Few non-related clusters
- Cases in related people separated by time and distance
- Person to person transmission amongst relatives (not unrelated contacts & HCWs)
## Family clusters of H5N1 cases

<table>
<thead>
<tr>
<th>Country</th>
<th>Total cases</th>
<th>No. of family clusters*</th>
<th>n/N (%) of confirmed cases occurring in family clusters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vietnam</td>
<td>101</td>
<td>12</td>
<td>24/101 (24)</td>
</tr>
<tr>
<td>Thailand</td>
<td>25</td>
<td>3</td>
<td>5/25 (20)</td>
</tr>
<tr>
<td>Cambodia</td>
<td>7</td>
<td>1</td>
<td>1/7 (14)</td>
</tr>
<tr>
<td>Indonesia</td>
<td>116</td>
<td>14</td>
<td>30/116 (26)</td>
</tr>
<tr>
<td>China</td>
<td>27</td>
<td>2</td>
<td>3/27 (11)</td>
</tr>
<tr>
<td>Turkey</td>
<td>12</td>
<td>3</td>
<td>8/12 (67)</td>
</tr>
<tr>
<td>Azerbaijan</td>
<td>8</td>
<td>2</td>
<td>7/8 (87)</td>
</tr>
<tr>
<td>Egypt</td>
<td>43</td>
<td>2</td>
<td>5/43 (5)</td>
</tr>
<tr>
<td>Iraq</td>
<td>3</td>
<td>1</td>
<td>2/3 (67)</td>
</tr>
<tr>
<td>Pakistan</td>
<td>1</td>
<td>1</td>
<td>1/1 (100)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>348</strong></td>
<td><strong>41</strong></td>
<td><strong>86/348 (25)</strong></td>
</tr>
</tbody>
</table>

*Cluster defined as at least two cases of clinically compatible illness with at least one case laboratory confirmed H5N1.

### Indonesia
- Karo cluster
- Siblings affected six months apart

### Multi-generation H2H in families
- Karo
- Pakistan
Conclusions

- Viral factors - ✓
  - but not whole story – does not explain patterns
- Dose / exposure intensity - X
- Site of inoculation / behaviour – ?
- Host factors - ????

Gaps

- Host factors

Recommendations

- Look at host phenotype
  - Antibody and CMI (?)
  - Receptors
  - Gene expression (mild vs severe H5 vs seasonal)
- Look for evidence of genetic effect
  - Increased familial relative risk
  - Genetic studies
All do-able!

Design and methods

• Conduct a **linkage study** in families with multiple H5N1 cases to investigate if any single nucleotide polymorphisms (SNPs) are transmitted to affected offspring more or less often than expected;

• Conduct a genome-wide, **case-control association study** to investigate if any SNPs occur at a significantly different frequency in affected individuals compared to unaffected individuals.
Linkage study

- Use related individuals
- To identify regions of genome that contain genes predisposing to disease
- By looking for marker loci transmitted from parents to affected offspring more often than expected.
- Since loci close to each other on the same chromosome are more likely to be inherited together than loci far apart
- The marker loci should be located near the gene predisposing to disease

Linkage study

- Assemble pedigrees of multiply affected families
- Ascertain availability of DNA
- Obtain consent and specimens
- Genotype with genome-wide linkage panel of 10,000 SNPs
- Undertake linkage analysis, looking for regions of the human genome inherited with the disease state by calculating parametric and non-parametric logarithm of the odds (LOD) scores.
Association study

- Use unrelated individuals
- To identify regions of genome that contain genes predisposing to disease
- By looking at the frequency of genetic markers in cases and controls
- Markers that appear more frequently in cases compared to controls may be located near the gene predisposing to disease

Association study

- Identify suitable cases
- Proceed if sufficient cases can be enrolled
- Identify suitable control groups
  - For Vietnam – community cohort or poultry cullers or cord blood controls
- Genotype using a genome-wide genotyping kit of more than 550,000 SNP loci
- Calculate odds ratio, chi-square test statistic, Armitage trend and p values for the association between individual SNP genotype and disease status
Genome-wide not candidate gene approach

Few data on genetic determinants of influenza infection and disease.
Possible candidate genes include:
- Cell surface sialic acids recognised by viral haemagglutinin
- MX1 gene (interferon induced factor)
- Toll like receptors
But little solid data to support any choice.