WHO Process for Monitoring Novel Influenza Viruses

FAO-OIE-WHO Joint Technical Consultation on Avian Influenza at the Human-Animal Interface
Verona, 7-9 October 2008

WHO Global Influenza Surveillance Network (GISN)
(Established in 1952)

5 WHO Collaborating Centres (Atlanta, London, Melbourne, Tokyo, Memphis)
~120 National Influenza Centres (>80 countries) (gaps!)

Main Objectives:
• Monitor - epidemiology of influenza and burden of disease
  - antigenic/genetic changes in circulating A and B viruses
  - spread of antigenic variants
  - emergence and persistence of drug resistance
  Make biannual recommendations on vaccine composition
• Early detection of novel human viruses, assessment of pandemic risk
  - identify the virus (genetic/antigenic) – new human subtype?
  - identify source of infection and extent
  - sporadic or local clusters of infection (serological evidence)
  - human-human transmission?
  - geographical spread
  - develop candidate vaccine strains
Monitoring emergence of human virus with pandemic potential (characteristics)

Identity of the virus (genetic/antigenic) – new human subtype?

- Swine H1N1, H1N2, H3N2 - antigenically different from human subtypes (not considered likely pandemic threat)
  - important to monitor changes among swine viruses
  - to readily identify source of infection (e.g. A/HK/1774/99, H3N2)
  - emergence of novel subtypes (e.g. H2N3) with increased pandemic risk

- Avian viruses:
  - H7N7, H7N3, H7N2 - mainly mild infections, conjunctivitis
    - little change (animal/human)
    - source of infections removed
  - H9N2 - mild infections; partial human receptor-binding characteristics
    - widespread
  - H5N1 - highly pathogenic, diverse genetically and antigenically
    - increasing spread

The HA of H9N2 virus, with leucine 226, has preference for the human-like (α2,6-linked sialic acid) receptor - intermediate in human adaptation? - does it pose a greater pandemic threat?
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Monitoring changes in novel human influenza viruses

Genetic changes/increased diversity (all genes):

- In source viruses - genetic reassortment (avian/human)?
  - increase human infection (pandemic risk) [PB2 E627K of clade 2.2]
  - change in clinical outcome?
  - drug resistance (established mutations)?

- Following animal to human transmission - adaptive changes? (increase human transmission)
  - HA receptor binding (increased preference for human receptors)
  - Polymerase activity, e.g. PB2: E627K, D701N
  - Altered virulence?, e.g. NS1, PB1- F2

Antigenic changes/increased diversity (using ferret post-infection antisera):

- Diagnostics
- Vaccines: update of candidate strains?
  - cross reaction/protection
- Cross-reactivity of antibody responses to natural infection

Resistance to anti-M2, anti-NA drugs :

- Effectiveness of antivirals (stockpiles)
Changes in HA of H5N1 following avian to human transmission

Yamada et al, 2006

Monitoring changes in novel human influenza viruses

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    (WHO Working Group on PCR Protocols)
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Amino acid differences between clades/subclades of H5 haemagglutinins (HA)

Figure 1. Phylogenetic relationships of H5N1 viruses.
Monitoring changes in novel human influenza viruses

Antigenic changes/increased diversity (using ferret post-infection antisera): Diagnostics
Vaccines: update of candidate strains?
- cross reaction/protection
Cross-reactivity of antibody responses to natural infection?

### Table 1. Antigenic properties of H5N1 viruses

<table>
<thead>
<tr>
<th>REFERENCE ANTIHEMAGGLUTINATION TITERS</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
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<td>128</td>
<td>64</td>
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Clade

- 1
  - 2.1
  - 2.2
  - 2.3
  - 2.4
  - 2.5

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**Phylogenetic comparison of the M genes of H5N1 viruses**

- Presence of mutations (V27A, S31N) in the M2 protein confer resistance to amantadine and rimantadine
  - Clade 1 resistant
  - Clade 2.1 ~80% resistant
  - Clade 2.2, 2.3 sensitive
Effect of Oseltamivir Treatment on Virus Load in H5N1 Patients

M. de Jong et al.  N. Eng. J. Med. 2005

Phylogenetic comparison of N1 neuraminidase genes
Proximity of recent amino acid changes in NA of H1N1 viruses to the catalytic site

Structural basis for difference in Oseltamivir-resistance determinants

Arg292Lys(N2)
Tyr347 in N1 (green) compensates for loss of H-bond by R292K mutation

His274Tyr(N1)
Conformation of 270 loop and smaller residue at 252 in N2 (yellow) accommodate larger tyrosine in N2
Effects of the H274Y mutation on the location of Glu 276 of N1 of A/Vietnam/1203/04(H5N1) in complex with oseltamivir or zanamivir

Wild type (yellow); H274Y mutant (green)

Effect of the Asn294Ser mutation on oseltamivir binding to N1 of H5N1

Km 8 x wt
Ki (Zam) 7 x wt
Ki (osel) 81 x wt
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