Influenza Virological Surveillance and Genotyping

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Rationale for virological influenza surveillance

- Influenza viruses differ in
  - their antigenic properties,
  - the age/risk groups they affect,
  - their pathogenicity,
  - their susceptibility to antiviral drugs.
- The viruses also constantly and rapidly evolve.
- Furthermore, the symptoms of influenza virus infection overlap with other infections.
  - Laboratory verification is therefore an essential complement to any epidemiological influenza surveillance scheme.
Scope

• Objectives of virological influenza surveillance,
• How it is organised in order to achieve
  – a representative sampling of circulating viruses,
  – as well as ability to rapidly
    • detect,
    • understand,
    • respond to emerging changes.
• The relevant
  – virus characteristics
  – laboratory methods used
• Current and future opportunities and challenges for the virological surveillance of influenza
Objectives of virological influenza surveillance

- ILI and verification of real flu
- Occurrence of types and subtypes
- “True epidemiology”
- Monitoring evolution, drift and shift
- Providing viruses, vaccine antigen, reagents
- Maintaining effective diagnostics
- Early warning
Objective: ILI and verification of “true flu”

- Usually, but not always good correlation

**Norway - Season 2010 / 2011**
ILI consultations per 100,000 population and influenza positive specimens

**Belgium - Season 2010 / 2011**
ILI consultations per 100,000 population and influenza positive specimens

Source: The WHO European Influenza Network (EuroFlu.org)
Compiled at 11:27 on May 12 2011
• ILI peak in week 35-36 was not supported by virological surveillance
• 'Hypersensitivity' due to public and professional concern?
• A rhinovirus epidemic took place in August-September
Objective: Occurrence of types and subtypes

- Monitor circulation of all relevant types/subtypes (and lineages)
  - Type A, B
  - Type A: subtypes H1(pre-2009 seasonal & 2009 pdm), H3, …
  - Type B (Vic/2/87 & Yam/16/88 lineages)
- Unbiased surveillance algorithm
  - Balance in part upset during/after 2009 pandemic
- Timely for usefulness
Objective: “True epidemiology”

- Understanding the “true epidemiology” is necessary for optimal response and prevention.
- Genetic analysis of the very heterogeneous influenza viruses allows dissecting the relationships between viruses (and thus infections) in great detail.
- Demonstrates that a flu outbreak can consist of many sub-epidemics.
- Some apparent patterns (e.g. spread of epidemic between neighbouring countries) can be refuted by virus genotyping evidence.
Objective: Providing viruses, vaccine antigen, reagents

- Northern hemisphere flu season often comes underway only in January/February
- Things must be in place in February to have vaccine for the next season
Process of influenza vaccine virus selection and development

Seasonal

1. Collection of specimens and disease/epidemiological data (all year round)

2. Diagnosis, virus isolation in MDCK, preliminary analysis (hours -3 weeks)

3. Ferret antisera production (3-5 weeks)

4. Thorough antigenic and genetic analysis (1-3 weeks)

5. Review and selection of candidate viruses for vaccine use

6a. Classical reassortment of high-growth viruses for H1N1 & H3N2 (3-4 weeks)

6b. Reassortment of high-growth viruses using reverse genetics (and full safety testing) (6 weeks)

7a. Antigenic and genetic characterization of reassortants (4 weeks)

7b. Antigenic and genetic characterization of reassortants (4 weeks)

8. Development of standardized reagents for inactivated vaccines (6 weeks)

9a. Development of standardized reagents for inactivated vaccines (3 weeks)

9b. Development of standardized reagents for inactivated vaccines (6 weeks)

Availability of vaccine viruses and standardized reagents

W Zhang, WHO
The 2002/2003 Season – an example of role for genetic analysis at national level

- Incidence of ILI remains low, but a rising trend is apparent in week 7
- Sporadic circulation of A(H3N2) since mid-January; early detections mostly in hospitalised infants
- Sporadic influenza B since Late December

[Graph showing incidence of influenza-like illness (ILI) over time with regions highlighted for specific data points.]

<table>
<thead>
<tr>
<th>Week</th>
<th>Region Øst (East)</th>
<th>Region Sør (South)</th>
<th>Region Vest (West)</th>
<th>Region Midt (Middle)</th>
<th>Region Nord (North)</th>
<th>Hele landet/ Country total</th>
</tr>
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<tbody>
<tr>
<td>Uke/Week</td>
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</table>
Viruses from the 2002/2003 season are in **bold**; reference/vaccine strains are underlined.

Aligned partial HA gene sequences, neighbour-joining of Kimura-corrected genetic distances. The genetic distance between two strains are represented as the sum of the length of horizontal branches connecting them.

1% substitutions per site
The reporting of novel patterns

Differences in HA1 antigenic sites - emergence of Fujian/411/02 drift variant

Differences in HA1 antigenic sites

First Fujian/411/02-like identification in Europe, January 2003:

A/Oslo/6706/2001 (H3N2)
HA compared to A/Panama/2007/1999

Ser186>Gly
Asn144>Asp

Red - Antigenic site A
Orange - Antigenic site B

A/Oslo/613/2003
HA compared to A/Panama/2007/99

Receptor binding

A
B
C
D
E
Part of a long chain

- Chain of events from the patient all the way to successful vaccination involves many others:
- Surveillance, and provision of the right vaccine, is only helpful of the original and the ultimate elements function!
Objective: Maintaining effective diagnostics

- Influenza viruses (can) evolve rapidly
- Antigenic, functional, and genetic changes can affect the functionality of tests and protocols
  - Antisera/mAbs can fail
  - PCR primers and probes can fail
    - Review sequence match continuously
    - Good to have ‘fall-back’ alternative protocols
  - Substrates in functional tests can fail
    - RBCs in HA / HI testing (particularly recent H3N2 viruses)
Objective: Early warning
Picking up novel developments

Requirements / success factors

• Have an eye for new developments
• Do analyses timely
• Keep in touch with network and reference laboratory
• Follow up research
The relevant virus characteristics

- Antigenic characteristics
- Genetic makeup / relatedness to other virus strains
- Fitness (ability to replicate and spread)
- Pathogenicity
- Susceptibility to antiviral drugs
- Suitability to be captured / analysed by diagnostic tests and characterisation protocols in use
Desirables

• Representativeness
  – Geographical
  – Representative over time
  – Age groups
  – Different patient categories

• Adequate analysis
  – Routine for all; comprehensive for some

• Timeliness
  – Information and materials in time to be useful for public health
Genetic characterizations in different periods of the season 2010/11

Genetic characterizations Oct 2010

- swjH 1/California/7/2009
- swjH 1/Christchurch/16/2010
- swjH 1/England/142/2010
- H3-Pettr 16/2009
- H3-Victoria/208/2009
- H3-Victoria/208 subgroup HK/2121/2010
- BvIC-B/Brisbane/60/2008
- BvIC-B/B’des h’s333/2007
- DvIC-CladeB/Florida/4/2006

Genetic characterizations Oct-Dec 2010

Genetic characterizations Oct 2010-Mar 2011

Genetic characterizations Oct 2010-May 2011
Surveillance organised to achieve

- Public Health usefulness ("Information for Action")
- Representative sampling of circulating viruses,
  to achieve
  - Early detection of emerging changes
  - Rapid understanding of emerging changes
  - Timely and adequate response to emerging changes
The WHO-coordinated Global Influenza Surveillance Network

- has been in operation since the late 1940s, when the need to monitor the rapid changes in the virus in order to provide up-to-date, efficacious influenza vaccine was discovered.

- National Influenza Centres collect virus-containing specimens, identify influenza viruses, report their findings, and forward representative virus specimens or isolates to WHO Collaborating Centres.

- CCs perform detailed characterisation, particularly on the antigenic properties of the viruses.

- One important outcome of this process is the twice-yearly WHO recommendations for the composition of influenza vaccines, together with the virus strains needed for vaccine production.
Growth of WHO Global Influenza Surveillance Network (GISN)

- **1962**
  - 2 International Centres
  - 59 National Influenza Centres

- **1984**
  - 3 Collaborating Centres
  - 108 NICs in 76 countries

- **2010 (June 14)**
  - 5 Collaborating Centres (plus Australia and Japan)
  - 134 NICs in 104 countries
  - 4 Essential Regulatory Laboratories
  - Ad-hoc laboratory groups: e.g. H5 Reference Laboratories, PCR Working Group

WHO Informal Consultation on Improving Influenza Vaccine Virus Selection
W. Zhang • 14 June 2010 • Geneva
Regional integration

- WHO EUR – EuroFlu
- EU/EEA – ECDC/CNRL
  - EQAs; Task Groups; Training courses; reagent support; guidance; harmonisation etc.
- Increasing coordination in Europe
- Also increasing integration in other regions
Influenza virological surveillance at national level (Norway)

Norwegian laboratories (ca 20)

Sentinel physicians ("fyrtårn") (ca 70)

Weekly reports

Samples with virus

Sample result

Samples from patients with influenza symptoms

Viruses from Norway

NIPH

Weekly reports

WHO ref.lab

WHO

ECDC

Vaccine virus etc.

www.fhi.no

WHO

ECDC
Laboratory methods used in influenza surveillance

- Virus isolation in mammalian cells or embryo-nated eggs (*NIC; CC*)
- Identification / Antigenic characterisation through haemagglutination-inhibition, virus neutralisation, SRH.. (*NIC; CC*)
- Viral RNA detection and identification by RT-PCR (*prim.lab; NIC; CC*)
- Genetic characterisation by sequence analysis (*NIC; CC*)
  - Conventional (Sanger) sequencing; focused genotyping (pyrosequencing/SNP PCR etc); “Next-Generation Sequencing”
More on lab methods

Norway & other countries

- Epidemics differ!
- Virol. surveillance must have good geographical coverage
- Different epidemics leave different population immunity
- thus the susceptibility will differ in the next season
  - Likely that geographical differences will continue
Monitoring B lineages

- Distinct and diverging lineages since the 1970s(?)
- Recognised in late 1980s
- B/Victoria/2/87 and B/Yamagata/16/88
- Vic-lineage absent from global scene cca 1991-2001 (but present in east Asia)
  - Re-emerged 2002 and has co-circulated since
- Defined by HA genetic lineage
  - Genetic reassortment has occurred repeatedly, viruses are lineage ‘mosaics’
Flu B HA phylogeny

- Evolution toward subtypes?
B virus characterised by variant, 2005/06, by patient age

- The most numerous virus may not be the one affecting risk groups the most
Monitoring flu B lineages

• Best possible information needed for risk assessment and vaccine composition recommendations
• Different age distribution
• WHO FluNet database captures B virus lineage data,
  – But limited data entered
• Surveillance should provide adequate and timely information
  – Traditionally discriminated by antigenic characterisation of cultured virus (takes time)
  – Molecular rapid tests have been missing – but are coming now!
    • Real-time duplex RT-PCR, conventional PCR, others..
• Weekly up-to-date information should be feasible
Norwegian influenza serosurveys have been carried out since the late 1970’s

Established just in time to record the previous H1N1 "pandemic" in 1977-78

Leftover sera from hospital labs
- All-country coverage
- age representative
- anonymous, only sex, age, sampling time (approx.) and county of residence
- Collected annually in August

Testing for antibody reactive to relevant virus variants by HI

Outcomes reported to WHO Vaccine Composition Meeting
Pre- and Post Pandemic immunity

- Substantial increase in all age groups
- Highest in age below 20
  - Highest recorded incidence
  - High vaccine uptake
- Overall high seroprotection rate
- Some pre-existing immunity

Prevalence of immunity in January probably sufficient to provide “herd immunity”
Serological surveillance

HI reactivity to contemporary influenza B strains, sera collected 1992-2009

% Seropositive

Year

Predictive value of serosurvey

- Serum panel August 2010
- ”Wall of immunity”

Vic-lineage flu B was the weak link
Predictive value of serosurvey

- Serum panel August 2010
- "Wall of immunity"
- Vic-lineage flu B was the weak link

Influenza season 2010 - 2011
Predictive value of serosurvey

- Serum panel August 2010
- "Wall of immunity"

Vic-lineage flu B was the weak link

Influenza season 2010 -2011
Flu and predictions…

• People making predictions on flu is a well documented risk group for humiliation
Objective: Monitoring evolution, drift and shift
Virus evolution at RNA, protein, & antigen levels

Evolution at the nucleotide level is gradual; Resulting antigenic drift occurs in steps

Genetic analysis complements and adds to phenotypic analyses, but cannot replace them

- Genetic analysis does not measure directly the phenotypic trait
Genetic analysis can be more timely and ‘reliable’

• Antigenic analysis depends on reagents
  – Takes time to produce/provide
  – Output of analysis is particular to virus-reagent interaction
  – Can be hard to compare/interpret

• Genetic analysis relies on “universal” method and principle
  – Rapid and robust
  – Output (the sequences) is ‘absolute’ information
  – But genotype/phenotype relationships incompletely understood
Picking up changes in pathogenicity

• Determinants for pathogenicity to be covered later in the course (prof. Klenk)
• An example from Norway on picking up a candidate pathogenicity marker
Picking up changes in pathogenicity
Is this mutation changing receptor specificity?

HA  Asp222Gly
(pos 225 in H3)

- Documented for the 1918-virus
- Same effect in the 2009-virus?
Two 1918 HA variants
South Carolina with Asp 190 and Asp 225
bound exclusively to alpha-2,6-receptors

New York with Asp190 and Gly225
had mixed alpha-2,3/2,6 specificity

Only one mutation in the HA was sufficient to revert the HA receptor preference to that of classical avian strain.
Mutert virus har ikke smittet

Vaksinen holder. To av
menn som døde i pandemien
muterte viruset. Myndighetene
forstår at det trengs en ny type
vaksine.

Norway: Mutated H1N1 Swine Flu Detected by Scientists

Experts Say Swine Flu Mutations Do Not Warrant New Alarm

Virusendring skjer trolig i kroppen

Mutasjonen har trolig skjedd i
kroppen til noen av de funnet av
avvikelser som har blitt funnet
av helsemyndighetene. Men
helsemyndighetene vet ikke
hvordan dette skjer.

«Norsk» influensamutations tok to liv i


The New York Times

Nyheter

SMITTEFARE!

Respekt for de omkring i kontakt med.
Apparent pattern / Hypothesis

- The mutation is not in circulation
- The mutation occur sporadically
- Altered binding specificity and cellular tropism make the virus prone to infect a wider range of cells in the lower respiratory tract
- Correlation between occurrence of the D222G mutation and severe disease
Infection with the recently emerged pandemic influenza A(H1N1) virus causes mild disease in the vast majority of cases, but sporadically also very severe disease. A specific mutation in the viral haemagglutinin (D222G) was found with considerable frequency in fatal and severe cases in Norway, but was virtually absent among clinically mild cases. This difference was statistically significant and our data are consistent with a possible causal relationship between this mutation and the clinical outcome.

in any of 205 mild cases investigated (Table), thus the frequency of this mutation was significantly higher in severe (including fatal) cases ($p<0.001$, Fisher’s exact test, two-sided) than in mild cases. D222G mutants were detected throughout the sampling period, from the first recorded severe cases in July until early December. The frequency of another substitution in the same position, D222E, did not differ significantly between mild and severe cases ($p=0.772$). Yet another substitution, D222N, was observed in a very few cases ($n=1$), and at a higher rate than expected among severe
The difference in frequency of the mutation between the severe/fatal and mild cases is statistically highly significant \((p < 0.001, \text{Fisher’s Exact Test, two-sided})\) and indicates a strong correlation.

Consistent with hypothesis that this mutation may contribute to severe illness

But not neccessary for severe illness (and maybe not sufficient for severe illness?)

Apparently not well transmitted

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**Table**

Pandemic influenza A(H1N1) viruses characterised for amino acid position 222 of the haemagglutinin HA1 domain, by clinical outcome, Norway, May 2009–January 2010 \((n=266)\)

<table>
<thead>
<tr>
<th>HA1 position 222 genotype(^b)</th>
<th>Mild ((n=205))</th>
<th>Severe ((n=34))</th>
<th>Fatal ((n=27))</th>
<th>Severe plus fatal ((n=61))</th>
<th>All cases ((n=266))</th>
</tr>
</thead>
<tbody>
<tr>
<td>222D (wt)</td>
<td>92% (189)</td>
<td>82% (28)</td>
<td>59% (16)</td>
<td>72% (44)</td>
<td>88% (233)</td>
</tr>
<tr>
<td>222G</td>
<td>0% (0)</td>
<td>8.8% (3)</td>
<td>30% (8)</td>
<td>18% (11)</td>
<td>4.1% (11)</td>
</tr>
<tr>
<td>222E</td>
<td>7.3% (15)</td>
<td>2.9% (1)</td>
<td>7.4% (2)</td>
<td>4.9% (3)</td>
<td>6.8% (18)</td>
</tr>
<tr>
<td>222N</td>
<td>0.5% (1)</td>
<td>5.9% (2)</td>
<td>3.7% (1)</td>
<td>4.9% (3)</td>
<td>1.5% (4)</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

\(^a\) Clinical outcome based on patient information, assigned into categories by a medical specialist according to WHO guidance criteria [1].

\(^b\) Percentage of genotype within each clinical category is given, with number of cases per category in parentheses.
Mutant / “wild type” mixtures

- 222G more frequently seen in mixture with 222D (wild-type) than in pure form
- Pyrosequencing assay was used (picks up
Summary D222G

- The mutation is still only found in patients with severe illness
- The same mutation was not found in any of the 205 analysed cases with mild disease
- This mutation has been found sporadically throughout the period of pandemic virus circulation, since the first severe and fatal cases in the summer. There is no indication that the mutated virus has increased in frequency during this period.
- The mutation seem to occur sporadically in single cases, with no or little onward transmission (mixtures, ‘switch in serial samples)
- Since the mutation was not found in the majority of severe and fatal cases it is clear that non-mutated viruses also are capable of causing severe disease
- Further investigations still needed to clarify if this mutation alter the virulence and transmissibility of the pandemic influenza A (H1N1)
Published papers on the topic since 2009:


Picking up novel developments

- Have an eye for new developments
- Do analyses timely
- Keep in touch with network and reference laboratory
- Follow up research
Current and future opportunities and challenges for the virological surveillance of influenza

- Sustainability / political/financial support
- Disconnection from the primary diagnostic setting
  - Bedside rapid tests..
  - Unsuitable specimen sampling format
  - Inadequate info collected and shared
- Imbalanced testing yields biased surveillance data
  - Worse after 2009 pandemic
  - Many test for H1 but not H3
  - “Testing algorithm chaos”
  - Even some labs test for A but not B
  - Representativeness of materials received from primary lab is unclear
- Lack of trust and virus sharing
- Communication failure
- Alienation
- Incompetence
  - Disconnection between public health/surveillance workers and academic research
Long-term prospects

• Will primary diagnostics be entirely “privatised”?
  – No feed into surveillance?

• Global / regional “super-labs” and less involvement of national level labs?
  – Potential for very powerful and fast virologic analysis
  – Loss of local considerations – loss of capability to translate the surveillance output into nationally/locally specific/relevant response
Thanks, and greetings from the National Influenza Centre, NIPH, Oslo, Norway

http://www.fhi.no/influenza

- Grethe H. Krogh
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