The European Surveillance Network for Influenza in Pigs (ESNIP): a different approach to influenza surveillance

Kristien Van Reeth, ESNIP 2 coordinator

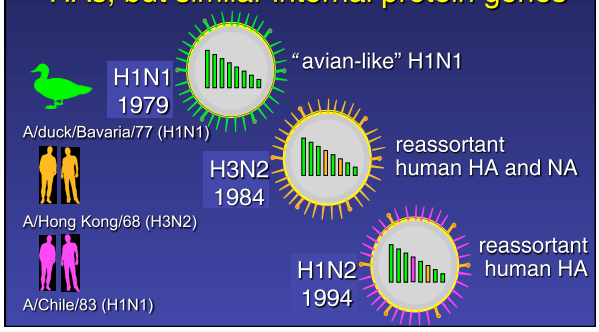
Outline

- History of ESNIP 1 (2001) and ESNIP 2 (2006)
- Major aims of ESNIP 2 and this symposium
- Limitations and considerations

About swine influenza viruses (SIVs)

- Three influenza A virus subtypes are enzootic in pigs throughout the world: H1N1, H3N2, H1N2
  but antigenic and genetic differences with human viruses
- Major cause of acute respiratory disease in pigs, but most infections are subclinical
- SIVs evolve at a much slower rate than their human counterparts, but some extent of antigenic drift may occur
- Genetic reassortment occurs frequently in pigs

SIVs in Europe have antigenically distinct HAs, but similar internal protein genes



About swine influenza viruses (SIVs)

- Diagnosis: no recommendations regarding reagents used in classical tests for serology (H1N1, H3N2 and H1N2 strains in HI) and SIV subtyping (hyperimmune sera)
  No kits with diagnostic reagents (↔ human flu)
- Vaccine manufacturers: No updates of strains, lack of information about antigenic characteristics of SIV field strains *No rapid procedure for replacement of strains*

Commercial SIV vaccines in Europe

Name (company)	Virus strains	Type	Antigenic dose
Gripovac	A/New Jersey/8/76 (H1N1)	split	H1N1 : ≥ 1,7 HI units
(Merial)	A/Port Chalmers/1/73 (H3N2)		H3N2 : ≥ 2,2 HI units
Suvaxyn Flu	A/sw/Netherlands/25/80 (H1N1)	whole virus	H1N1 : 4 µg HA
(Fort Dodge)	A/Port Chalmers/1/73 (H3N2)		H3N2 : 4 µg HA
Gripork	A/sw/Ollost/84 (H1N1)	whole virus	H1N1 : 3 x 10^7 EID_{50}
(Hipra)	A/Port Chalmers/1/73 (H3N2)		H3N2 : 2,5 x 10^7 EID_{50}
Respiporc Flu[1]	A/sw/Belgium/230/92 (H1N1)	whole virus	H1N1 : ≥ 256 HA units
(IDT)	A/sw/Belgium/220/92 (H3N2)		H3N2 : ≥ 256 HA units

All vaccines are inactivated vaccines with oil adjuvant or oil+ aluminium hydroxide
[1] registered only in Germany

Vaccine 19 (2001) 4452–4464

Antigenic and molecular heterogeneity in recent swine influenza A(H1N1) virus isolates with possible implications for vaccination policy

J.C. de Jong [a,d,*], P.P. Heinen [b], W.L.A. Loeffen [c], A.P. van Nieuwstadt [b], E.C.J. Claas [d,1], T.M. Bestebroer [a,d,2], K. Bijlsma [a], C. Verweij [a], A.D.M.E. Osterhaus [d], G.F. Rimmelzwaan [d], R.A.M. Fouchier [d], T.G. Kimman [a]

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[d] National Influenza Centre, Department of Virology, Erasmus University Rotterdam, P.O. Box 1738, Dr Molewaterplein 50, 3015 GE, Rotterdam, The Netherlands

! No harmonization/standardization of protocols and reagents used for antigenic characterization

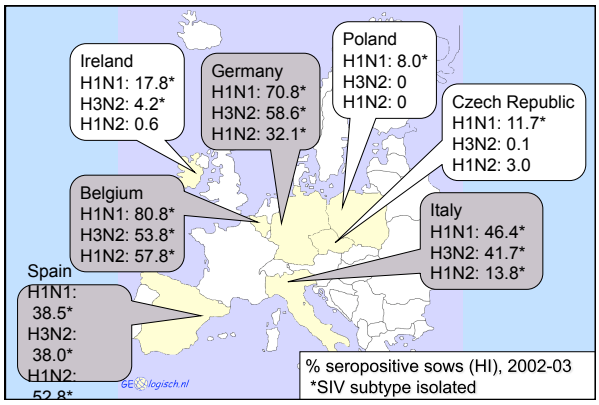
ESNIP 1

- Concerted Action, Jan 2001 - March 2004
- EC contribution 269 984 Euros
- Coordinator Guus Koch, CIDC-Lelystad, The Netherlands
- 14 partners from 10 European countries including 2 reference labs for human influenza, 3 industrial partners

Major realizations of ESNIP 1

- Standardization of protocols for SIV isolation in eggs and HI test
- Selection/production of reference virus strains and hyperimmune sera* that can be used for subtyping of European SIVs
- Establishment of a central virus bank with 23 isolates* from various regions in Europe
- Antigenic and genetic characterization of some SIV isolates

*available to non-ESNIP members on request



Seroprevalence of H1N1, H3N2 and H1N2 influenza viruses in pigs in seven European countries in 2002–2003

Kristien Van Reeth,[a] Ian H. Brown,[b] Ralf Dürrwald,[c] Emanuela Foni,[d] Geoffrey Labarque,[e,1] Patrick Lenihan,[e] Jaime Maldonado,[f] Iwona Markowska-Daniel,[g] Maurice Pensaert,[a] Zdenek Pospisil,[h] Guus Koch[i]

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- High infection rates with human-like H1N2 SIV
- Bivalent (H1N1+H3N2) SIV vaccines do not cross-protect against H1N2 in vaccination- challenge studies

Van Reeth et al., Vet Rec 2003

⇒ Inclusion of H1N2 in vaccines recommended

ESNIP 2

- Coordination Action, Jan 2006-Dec 2008
- EC contribution 300 000 Euros
- Coordinator Kristien Van Reeth, UGent, Belgium
- 9 partners from Europe
  including 2 SIV vaccine manufacturers
  1 partner from US, 1 from Hong Kong

ESNIP 2: important aims

1. Continue virological and serological surveillance for SIVs in Europe
   Lectures Wed June 4 (am)
2. Greater global interactions and a worldwide understanding of the epidemiology of SI *Lectures Wed June 4 (pm)*
3. Contribute to a more rational public health risk analysis of influenza in swine

SIVs in North America have a different history/origin than their European counterparts

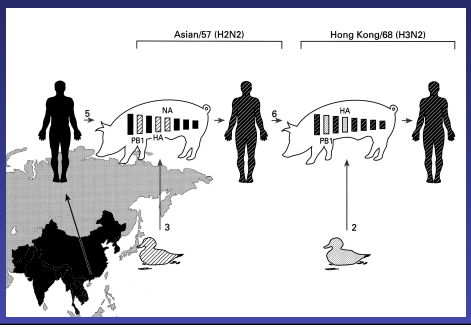
- Until 1998: only "classical" H1N1, descendant of 1918 Spanish flu virus
- Since 1998: reassortant (r)H3N2 viruses with genes of classical swine, human (HA and NA) and/or avian origin
- 1999: emergence of rH1N2 and H1N1, considerable antigenic diversity reported

"It appears that although a different series of introductions and reassortments have occured in the US, the epidemiology of SI in the US is now as complex and dynamic as that in Europe" Amy Vincent

SI situation in Asia appears to differ between countries

- SIVs circulating in Korea, China, Japan, Taiwan, Thailand
- South China: classical swine H1N1 (cf US) human-like H3N2
- South China is an "influenza epicenter": human pandemic viruses 1957, 1968 HP avian H5N1 1997, 2003

Human pandemic influenza viruses: reassortment is postulated to have occurred in the pig



Few comparative studies of SIVs and SI epidemiology in different parts of the world

- ? How different is SI epidemiology in South China compared to Europe/US
- ? Antigenic cross-reactivity between SIVs from different continents
- ? Heterogeneity in NA / internal proteins

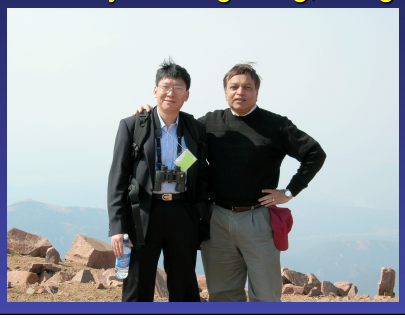
ESNIP 2: Include SIVs from US/South China in antigenic/genetic analysis of European SIVs. Exchange data on virus circulation.

ESNIP Partner 11: Chris Olsen, School of Veterinary Medicine University of Wisconsin - Madison, US

Chris Olsen

- Genetic characterization of H3N2 influenza viruses isolated from pigs in North America, 1977-1999... *Virus Research 2000* Genetic characterization of H1N2 influenza A viruses isolated from pigs throughout the US *J Clin Microbiol 2002*
- Isolation and characterization of H4N6 avian influenza viruses from pigs with pneumonia in Canada *J Virol 2000* Characterization of a swine-like reassortant H1N2 influenza virus isolated from a wild duck in the US *Virus Research 2003*
- Restricted infectivity of a human-lineage H3N2 influenza A virus in pigs is HA and NA gene dependent *J Clin Microbiology 2006*

ESNIP Partner 10: Malik Peiris, The University of Hong Kong, Hong Kong



Malik Peiris

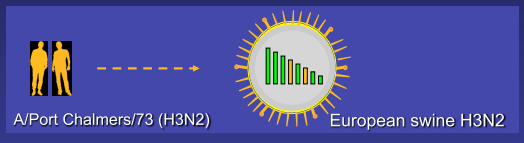
- WHO H5 reference laboratory
- genetic and antigenic characterization of ≥ 250 H5N1 isolates (1997-present)
- discovery of SARS-coronavirus in 2003

- "cytokine storm" may account for unusual severity of H5N1 in humans
- influenza surveillance of pigs at central abattoir in Hong Kong (80% of pigs from 9 provinces in mainland China)

ESNIP 2: important aims

1. Continue virological and serological surveillance for SIVs in Europe
   Lectures Wed June 4 (am)
2. Greater global interactions and a worldwide understanding of the epidemiology of SI
   Lectures Wed June 4 (pm)
3. Contribute to a more rational public health risk analysis of influenza in swine
   Lectures Thu June 5 (am)

Many SIVs have a human origin but human viruses change after introduction in swine population



1) Genetic reassortment:
   human HA, NA x swine/avian internal genes
2) Drift in HA: evolution of swine and human H3N2 viruses compared to Port Chalmers/73 *(de Jong et al., J Virol 2007)*
   → genetic drift approx. similar
   → antigenic drift more than 6 times slower for swine viruses

The various influenza surveillance networks are usually focused on categories of host

? Antigenic cross-reactivity between current viruses from swine and humans
? Identity in NA and other proteins
? Frequency of reassortment in different animal species
? Patterns of circulation

ESNIP 2: Include human viruses in antigenic/genetic analysis of European SIVs. Attempt to establish interactions with other surveillance networks.

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(1) Conclusions about antigenic relatedness of influenza viruses are often a reflection of the reagents used in cross-HI tests

Example: Swine H1N2 viruses and their human parent viruses

	HI titre with			
	Hyperimmune **rabbit** sera		Post-infection **ferret** sera	
Virus	Sw/Fin/ 2899 /82	A/Bz/ 11/78	Sw/Fin/ 2899/82	A/Bz/ 11/78
Sw/Finistère/2899/82 (H1N1)	5120	40	1280	<40
A/Brazil/11/78 (H1N1)	80	5120	<40	640
A/Chile/1/83 (H1N1)	40	**5120**	<40	**80**
Sw/Scot/410440/94 (H1N2)	80	5120	<40	320
Sw/Italy/1521/98 (H1N2)	<40	**5120**	<40	**<40**
Sw/CA/604/99 (H1N2)	80	5120	<40	160
Sw/Italy/1081/00 (H1N2)	<40	**5120**	<40	**<40**
Sw/Italy/1654-1/99 (H1N2)	40	**5120**	<40	**40**

Marozin et al., J Gen Virol 2002

Example: Swine H3N2 viruses and their human parent virus

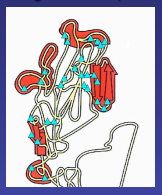
	Post-infection **swine** sera						
	A/PC/1/73		Sw/G/1/84		Sw/Fl/1/98		
Virus	Pig 1	Pig 2	Pig 3	Pig 4	Pig 5	Pig 6	Pig 7
A/P Chalmers/1/73 (H3N2)	80	160	40	40	**160**	**<40**	**<40**
Sw/Gent/1/84 (H3N2)	<40	160	640	160	<40	80	80
Sw/Flanders/1/98 (H3N2)	80	320	320	320	**160**	**1280**	**320**

Other pitfalls of cross-HI tests:

- Repeatability problems
- Disparate reactions
- Lack of symmetry between reactions

(2) The true significance of antigenic/genetic drift for vaccine efficacy can only be assessed by vaccination challenge studies in pigs

HA of A/New Jersey/8/76 (H1N1) vaccine strain compared to Sw/ Belgium/1/98 (H1N1)



Genetic analysis: only 72% aa identity, 28 aa changes in 5 antigenic sites !!

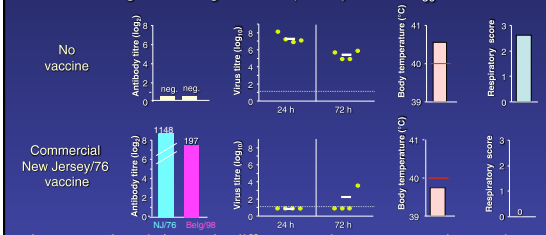
Cross-HI with post-infection swine serum

	HI antibody titre	
	A/NJ/76	Sw/B/98
A/N Jersey/8/76	160	<20
sw/Belgium/1/98	40	640

Vaccination - challenge study in pigs

Van Reeth et al., Vaccine 2001

Commercial vaccine based on A/New Jersey/8/76
Challenge: Sw/Belgium/1/98 (H1N1) $10^{7.5}$ EID_{50} intratracheal



Large antigenic/genetic differences between vaccine and challenge strains do not always result in lack of vaccine efficacy!

Lessons from vaccination-challenge studies in pigs

- Post-vaccination HI antibody titres to challenge virus correlate with protection against infection
- High homology between vaccine and challenge strains will not invariably result in high antibody titres, antigenic dose and adjuvant appear to play important roles
- Large variation between experiments and between pigs

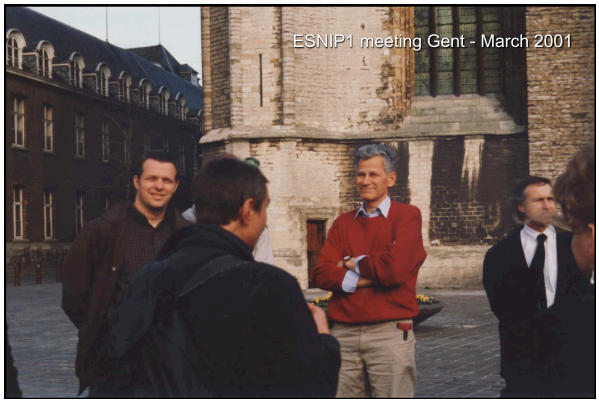
" Antigenic differences between vaccine and field strain can be overcome by a potent adjuvant and high antigenic dose"

(3) Important questions about the control of influenza in swine/humans must be addressed by experimental studies

- Extent of cross-protection between SIVs from different continents?
- Can immunity to older or more recent human H1 or H3 viruses (partially) protect humans against infection with swine viruses?
- Can pigs transmit avian influenza viruses to other mammals?

Surveillance...

- is essential for insights in the epidemiology and evolution of SIVs, as an alert for new viruses, to improve diagnosis ....
- is a building stone for influenza research
- may assist in vaccine strain selection, implementation of control measures, and contribute to determine public health risk




ESNIP 1 meeting Gent - March 2002


ESNIP 1 meeting Gent - March 2002


ESNIP 2 meeting Ploufragan - December 2007