



Laboratory of Virology
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Serological and virological surveillance during ESNIP 2, preliminary antigenic analyses

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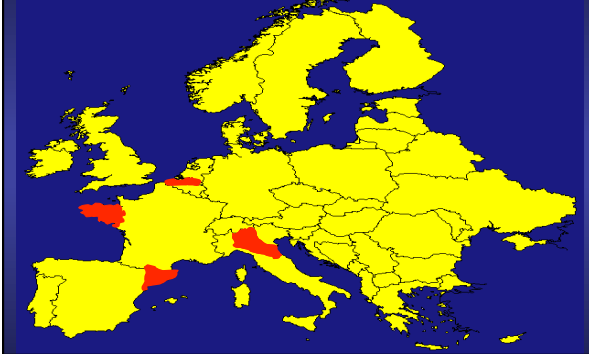
- Serological surveillance 2006-07
- Virological surveillance 2006-07
- Preliminary antigenic analyses
 - significance for serological diagnosis
 - significance for vaccination

Serological surveillance - Aims

To investigate:

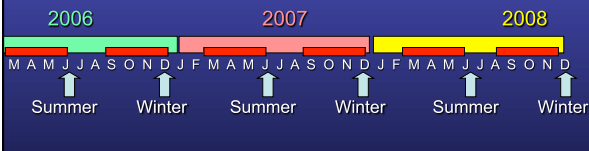
- the predominant SIV subtypes in major swine producing regions of Europe
- SIV subtype dynamics at farm level

Serological surveillance - Materials and Methods



Serological surveillance - Materials and Methods

- 20 farrow-to-finish farms
- NO influenza vaccination
- 10 sera from finishing pigs per farm
2 times a year (summer and winter)
for 3 years



Serological surveillance - Materials and Methods

Haemagglutination inhibition (HI) tests
against recent SIVs isolated in the area

Country	SIV strains used in the HI test		
	H1N1	H3N2	H1N2
Belgium	Sw/Belgium/1/98	Sw/Flanders/1/98	Sw/Gent/7625/99
Italy	Sw/Italy/1513/1/98	Sw/CA/3633/84	Sw/Italy/1521/98
France	Sw/Morbihan/0070/05	Sw/Flanders/1/98	Sw/Scot/41044/94
Spain	Sw/Finistère/82	Sw/Gent/84	Sw/Scot/41044/94

HI antibodies after infection with 2 subtypes

2 weeks after 2nd inoculation

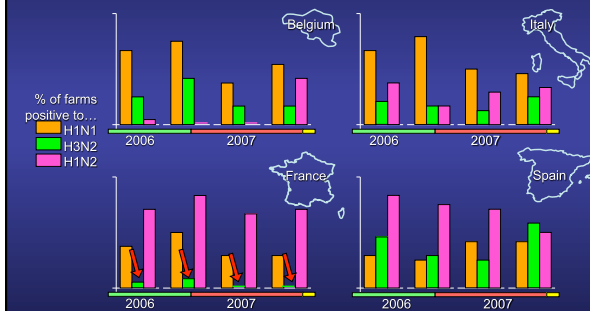
Virus inoculations	No pigs	No pigs with HI antibodies to...		
		H1N1	H3N2	H1N2
H1N1-4w-H3N2	6	6(80)	6(57)	0
H3N2-4w-H1N1	5	5(92)	5(61)	0
H1N1-4w-H1N2	5	5(211)	0	5(106)
H1N2-4w-H1N1	4	3(190)	3(13)	4(640)

Serologic cross-reactions in green

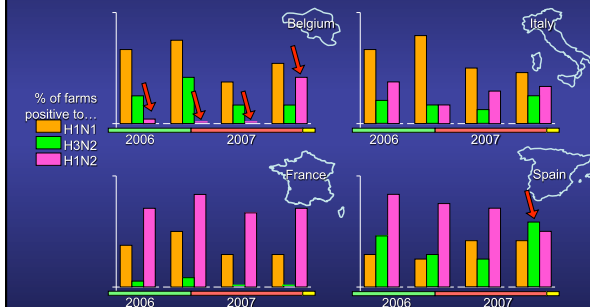
HI test discriminates between subtypes !!

(Van Reeth et al. 2006)

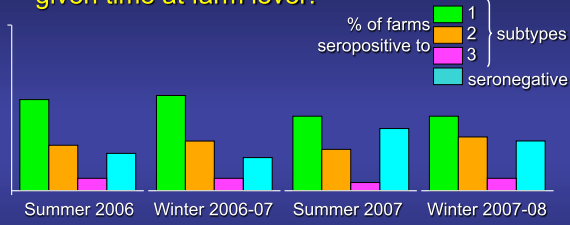
Q: How many SIV subtypes circulate at a given time at regional level?



Q: Do we have differences in the same region throughout the seasons examined?

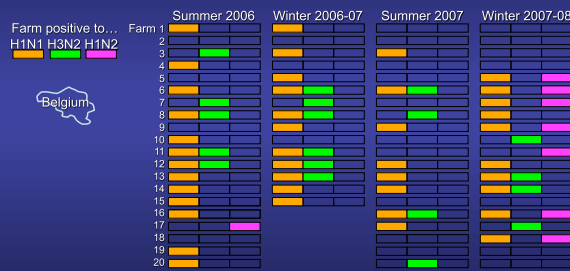


Q: How many SIV subtypes may circulate at a given time at farm level?

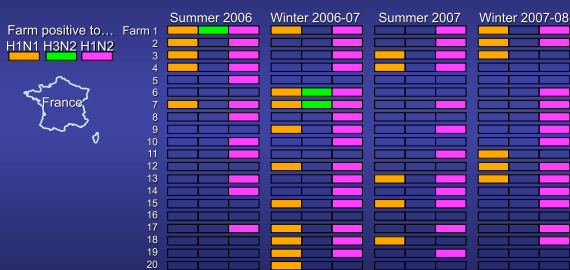


- 70% {
- 44% sero-positive to 1 SIV subtype
 - 26% to 2 SIV subtypes
 - 4% to 3 SIV subtypes
 - 26% sero-negative

Q: Do the same SIV subtypes continue to infect the same farm or is there an alternating pattern?



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Q: Do the same SIV subtypes continue to infect the same farm or is there an alternating pattern?

- No clear pattern of infection was identified:
a farm positive to a given SIV subtype in one period may be negative to the given subtype 6 months later
- However...
Belgium: 4 farms consistently seropositive to H1N1
Italy: 2 farms seropositive to H1N1
France: 5 farms seropositive to H1N2
Spain: 5 farms seropositive to H1N2 and 1 to H3N2

Serological surveillance - Conclusions

- Regional differences in the circulation of SIVs
- No repeating infection-pattern
- Circulation of the same SIV subtypes infecting some farms over the examined period may be the result of subtype prevalence in the region

More safe conclusions by the end of the survey (December 2008)

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Virological surveillance - Aims

- Collect as many SIVs as possible during a 3-year-period (2006-08)
- Monitor major changes in SIVs circulating in European pigs
- Select recent SIVs to include in the antigenic and genetic characterization

Virological surveillance - Materials and Methods

- Collection of clinical material (nasal swabs or lung tissue) from acute respiratory disease outbreaks
- Virus isolation in 10-day-old embryonated chicken eggs or cell culture (MDCK or Caco-3 cells)
- Subtyping of both haemagglutinin (HA) and neuraminidase (NA)

Virological surveillance - Materials and Methods

Partner, region under surveillance and method of HA and NA subtyping

Partner	Area	HA subtyping	NA subtyping
UGent	Flanders	HI test*	NI test*
VLA	England	HI test	NI test
IZSLER	Lombardia & Emilia Romagna	HI test and/or RT-PCR	RT-PCR
AFSSA	Brittany	HI test and/or RT-PCR	RT-PCR
HIPRA	Spain	HI test and/or RT-PCR	RT-PCR

* HI and NI tests performed against hyperimmune sera against Sw/Finistère/82 (H1N1) Sw/Gent/84 (H3N2) and Sw/Scot/41044/94 (H1N2)

Virological surveillance - Results

Overview of SIV isolates 2006-2007

Partner	Year	No of isolates	H1N1	H3N2	H1N2
UGent	2006	4	1	3	0
	2007	8	2	4	2
VLA	2006	7	6	0	1
	2007	4	4	0	0
IZSLER	2006	16	8	4	4
	2007	10	3	3	4
AFSSA	2006	22	12+1	0	9
	2007	25	13	0	12
HIPRA	2006	10	3	3	4
	2007	9	1	5	3
Total		115	54	22	39

Virological surveillance - Results

Effect of age group:

- 61%, from 3 to 6 months old pigs
- 22%, from adult pigs
- 17%, < 3 months old piglets

Effect of season:

- 28%, in Winter
- 27%, in Spring
- 20%, in Summer
- 25%, in Fall

NO SEASONAL EFFECT

9 SIVs isolated from vaccinated animals:

- 5 H1N2
- 2 H1N1 and 2 H3N2

Virological surveillance - Conclusions

- All isolates could be easily identified with hyperimmune sera against reference strains
- Novel H1N1 and H1N2 reassortants are relatively unimportant in the countries examined
(only 1 H1N1 reassortant virus isolated in France)
- Virological surveillance confirms the results of the serological surveillance

Detailed antigenic and genetic characterization of these SIVs is ongoing

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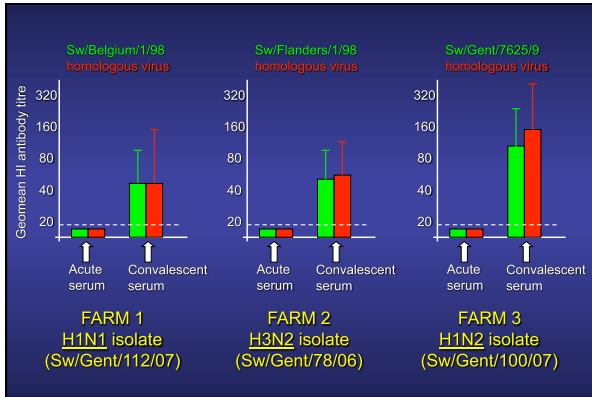
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Preliminary antigenic analyses: Serological diagnosis - Aims

- To compare the sensitivity of routinely used reference SIVs to the homologous farm isolate
- Need to update strains used in the HI test?

Preliminary antigenic analyses: Serological diagnosis - Materials and methods

- Collection of nasal swabs and/or lung tissue at every farm visit
- Tag animals and collect serum
- If we have a virus isolate on farm re-visit 3-4 weeks later and collect serum from the same pigs
- Perform the HI test using our 3 standard SIV strains (Sw/Belgium/1/98, Sw/Flanders/1/98 and Sw/Gent/7695/99)
+ farm isolate



**Preliminary antigenic analyses:
Serological diagnosis - Conclusions**

- Comparable antibody titres between farm isolate and SIV reference strains routinely used in the HI test
- No need to replace reference strains used by UGent

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Cross-reactivity of post vaccination sera Aims

- Do vaccines with more recent virus strains elicit higher HI titres against recent field isolates?
- Need to update vaccine strains?

Cross-reactivity of post vaccination sera Materials and methods

- Generation of post-vaccination sera using 3 commercial inactivated SIV vaccines containing:

Vaccine	Influenza virus strains
A	A/New Jersey/8/76 (H1N1)
	A/Port Chalmers/1/73 (H3N2)
B	A/Sw/Netherlands/25/80 (H1N1)
	A/Port Chalmers/1/73 (H3N2)
C	A/Sw/Belgium/230/92 (H1N1)
	A/Sw/Belgium/220/92 (H3N2)

Cross-reactivity of post vaccination sera Materials and methods



- HI test against recent field isolates:

H1N1:	H3N2:
Sw/Morbihan/0070/05	Sw/Spain/54008/04
Sw/England/33780/06	Sw/Gent/131/05
Sw/Italy/29313/06	Sw/Italy/10659/1/07
Sw/Gent/112/07	

Results: H1N1 SIVs

Strain	HI Ab titres of pigs vaccinated with...					
	NewJer/76		Netherl/80		Reas/92	
	Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2
NewJersey/8/76	640	160	40	20	80	10
Sw/Netherlands/25/80	320	80	160	40	160	20
Sw/Belgium/230/92	80	40	40	20	160	80
Sw/Morbihan/0070/05	80	20	10	<10	80	10
Sw/England/33780/06	640	80	40	20	80	40
Sw/Italy/29313/06	320	10	40	10	80	20
Sw/Gent/112/07	20	<10	40	<10	160	10

Results: H3N2 SIVs

Strain	HI Ab titres of pigs vaccinated with...					
	PortCh/73		PortCh/73		Reas/92	
	Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2
Port Chalmers/1/73	640	80	160	80	40	10
Sw/Belgium/220/92	80	10	80	10	320	80
Sw/Spain/54008/04	320	40	80	40	320	80
Sw/Gent/131/05	320	40	80	40	160	80
Sw/Italy/10659/1/07	320	40	80	40	320	80

Cross-reactivity of post vaccination sera
Conclusions

- Variation between pigs
- Vaccines containing more recent H1N1 or H3N2 SIV strains do not necessarily induce higher HI antibody titres
- Conclusions on vaccine efficacy with vaccination-challenge trials