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AGENCE FRANÇAISE
DE SÉCURITÉ SANITAIRE
DES ALIMENTS

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Genetic and antigenic characterization of swine influenza viruses in France: identification of novel H1N1 reassortants

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Pig production in France

- Typically farrow-to-finish units, family farms
- Average ~ 200 sows (increasing size)
- Usually one site (except some of the largest)
- Different compartments but high level of confinement
- **Brittany** = leading pig producing region (60%)
(7200 farms - 14,2 M pigs in 2006)





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Historical record of SIV in France (before 2000)

Dates	Virus type	Virus strains	Ab (HA) related to	References
-1973- early 1980s ?	Human-like swine H3N2	-	A/PC/73, A/VIC/75, A/Texas/77	Tillion, 1980 Aymard, 1980
From 1999		A/Sw/Finistere/127/99	A/Sydney/97	Marozin, 2002
? 1978	? Classical swine H1N1	-	A/NJ/76 (but not A/Sw/Iowa/31)	Tillion, 1980
1978-mid 1980s ?	Human-like swine H1N1	-	A/USSR/77	Aymard, 1980
From 1979	Avian-like swine H1N1	A/Sw/Finistere/2899/82 A/Sw/IleVilaine/1455/99	A/duck/Bavaria/77	Gourreau, 1980 Madec, 1983 Marozin, 2002
From 1984	Reassortant human-like swine H2N2 (hu-like sw H3N2 x av-like sw H1N1)	A/Sw/Cotes d'Armor/3633/84 A/Sw/Finistere/510/94	A/PC/73	Madec, 1984 Gregory, 2001

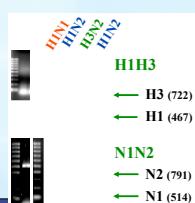
Historical record of SIV in France (before 2000)				
Dates	Virus type	Virus strains	Ab related to	References
1987-1988	Reassortant swine H1N2 (hu-like sw H3N2 x av-like sw H1N1)	A/Sw/France/5027/87	A/Sw/Finistere/82	Gourreau, 1994
From 1994	Reassortant human-like swine H1N2 (reassortant hu-like sw H3N2 x hu-like sw H1N1)	A/Sw/Cotes d'Armor/797/97 A/Sw/Cotes d'Armor/800/00	A/Sw/Scotland/94 A/USSR/77	Marozin, 2002

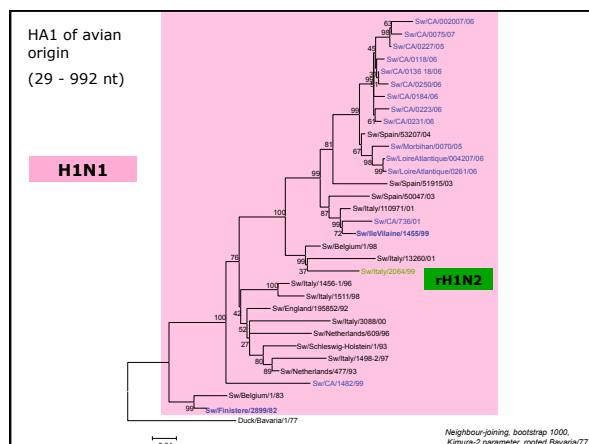
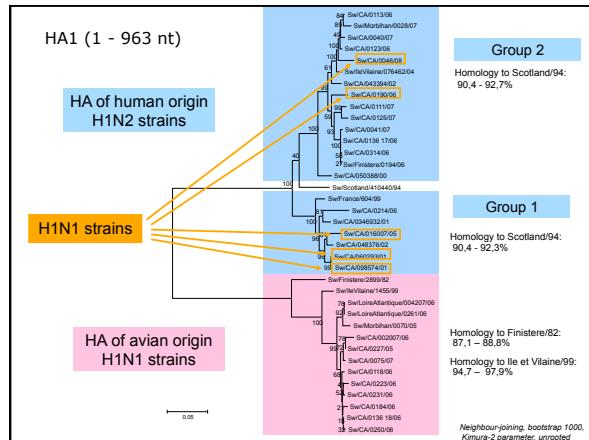
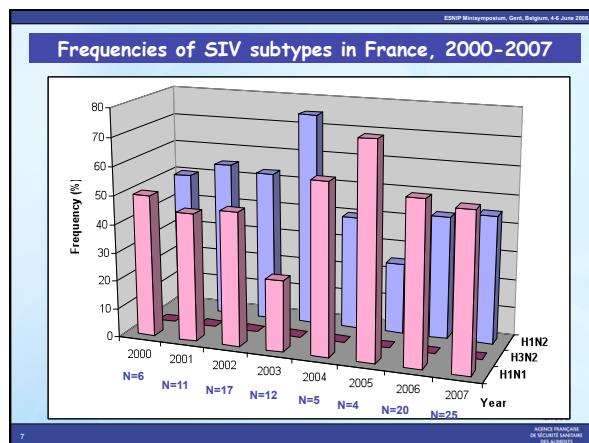
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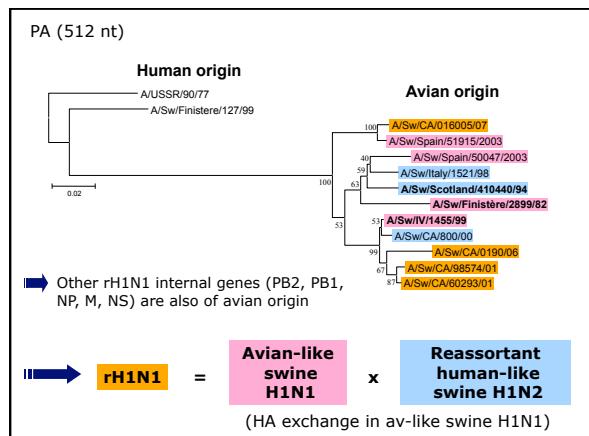
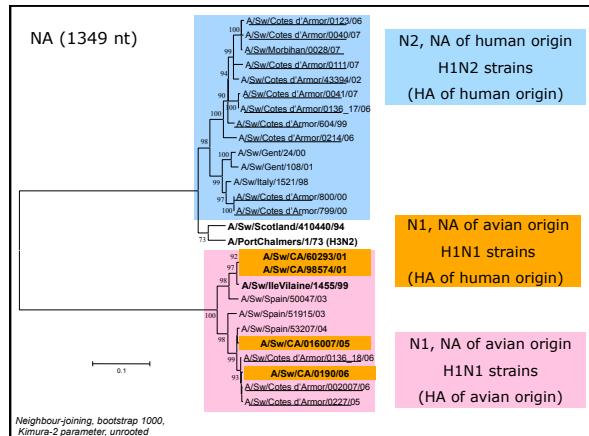
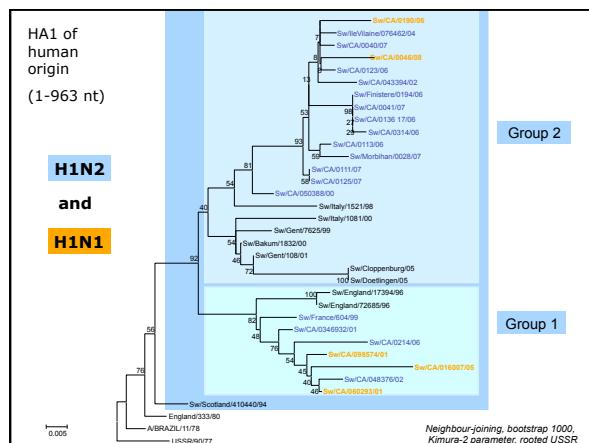

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Serological surveillance in France, 2001-2007				
1. ESNIP 1 (2001-2003): Brittany , 55 herds, 315 sera (Madec, 2004; van Reeth, 2004) 2. Fort Dodge (2006): Other regions than Brittany , 58 herds, 348 sera (Guillaume, 2007) 3. Fort Dodge (2006): Brittany , 62 herds, 492 sera (Labarque, 2008) 4. AFSSA (2006-2007): Brittany + Pays Loire + Basse-Normandie , 125 herds, 1875 sera (Kuntz-Simon, unpublished)				
H1N1 = dominant subtype				
H3N2 = no serological evidence for circulation (cross-reactive Ab) except in Nord-Pas-de-Calais				
H1N2 = circulation in Brittany and Basse-Normandie but not elsewhere				
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Subtype	Fatteners	Herds		
H1N1				
1. A/Sw/Bel/1/98	29 %	44 %		
2. A/Sw/Bel/1/98	24 %	41 %		
3. A/Sw/Bel/1/98	54 %	81 %		
4. A/Sw/Morbihan/0070/05	23 %	58 %		
H3N2				
1. A/Sw/Flanders/1/98	0 %	0 %		
2. A/Sw/Flanders/1/98	4%	7 %		
3. A/Sw/Flanders/1/98	6 %	13 %		
H1N2				
1. A/Sw/Scotland/410440/94	26 %	42 %		
2. A/Sw/Gent/7625/99	2 %	2 %		
3. A/Sw/Gent/7625/99	38 %	56 %		
4. A/Sw/Scotland/410440/94	34 %	54 %		

Sampling in affected herds and laboratory investigations				
> Acute respiratory disease: fever, loss of appetite, cough, ...				
E. Simon, AFSSA				
Commemoratives: vaccination, co-infections, age, movements...				
Nasal swabs in 10 individuals with hyperthermia > virus isolation: MDCK cells or embryonated eggs (+ isolates from LDA22) > HA test > sub-typing: double RT-PCR multiplex (Kuntz-Simon, 2005 ; Chiapponi, 2003) > HA and NA sequencing > partial sequencing of internal genes > phylogenetic analyses > antigenic characterization (HI test, NI test)				
Blood samples in the same 10 animals + 2nd blood samples three weeks later > seroconversion ascertainment: HI test				
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Antigenic characterization of French SIVs (2000 - 2007)							
HI antibody titers with hyperimmune swine sera to:							
Virus	Avian-like HA		Human-like HA				
	Ref strain	H1N1	rhH1N1	Ref strain	H1N2 C1	H1N2 C2	
Finistere 82	0070/05	0190/06	Scotland 94	0214/06	0113/06		
H1N1	20-160	320-1280	< 10	< 10	< 10	< 10	
H1N2 C1	<10-10	<10-20	10-40	320-1280	320-1280	160-320	
H1N2 C2	<10-20	<10-10	20-80	640-2560	320-640	320-1280	
6020/07 C1 98574/01 C1 016007/05 C1	<10-20	<10-40	< 10	80-320	320-640	40-160	
0190/06 C2	< 10	< 10	640	< 10	< 10	20	
0048/08 C2	10	20	<10	640	80	320	

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HI antibody titers with ferret serum to human H1N1		
Virus strain	Subtype (determined by multiplex RT-PCR)	human H1N1
Sw/Finistere/2899/82 - B	H1N1	<10
Sw/Scotland/421/04/094 - B	H1N2	160
Sw/Cotes d'Armor/0214/06 - B	H1N2 - G1	80
Sw/Cotes d'Armor/0434/02 - B	H1N2 - G7	20
Sw/Morbihan/0028/07 - B	H1N2 - G7	40
Sw/Cotes d'Armor/0113/06 - B	H1N2 - G2	80
Sw/Finistere/0194/06-M	H1N2 - G2	40
Sw/Cotes d'Armor/0111/07 - B	H1N2 - G2	40
Sw/Cotes d'Armor/0602/93/01 - B	rhH1N1 - G1	20
Sw/Cotes d'Armor/098574/01 - B	rhH1N1 - G1	80
Sw/Cotes d'Armor/016007/05 - B	rhH1N1 - G1	20
Sw/Cotes d'Armor/0190/06 - B	rhH1N1 - G2	<10
Sw/Cotes d'Armor/0046/08 - B	rhH1N1 - G2	nd

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Discussion (1)	
➤ No H3N2 virus isolated in Brittany (France) since 1999	
➤ Isolation of H1N1 and H1N2 viruses (~50/50)	
➤ Avian-like sw H1N1 isolates: all genetically and antigenically closely related	
➤ Reassortant human-like sw H1N2 strains: 2 genomic subgroups, but all antigenically closely related	
➤ Identification of novel reassortant H1N1 strains	
• hu HA + av NA + av internal genes	
• new evidence for co-circulation of H1N1 and H1N2 strains	
• 5 strains isolated from 2 farms	

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Discussion (2)

Farm « CAV » (Côtes d'Armor):
farrow-to-finish, no vaccination, outbreaks at 7 weeks of age

2000:	avian-like H1N1
2001 (spring):	reassortant H1N1 (G1)
2001 (autumn):	reassortant H1N1 (G1)
2002 :	reassortant human-like H1N2 (G1)
2005 (July):	reassortant H1N1 (G1)
2007 (March):	reassortant human-like H1N2 (G2)

3 strains rH1N1 genetically and antigenically closely related
evidence for the adaptation in pigs ?

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Discussion (3)

Farm « BAL » (Côtes d'Armor):
farrow-to-finish, 900 sows, vaccination of sows since 2004, most of outbreaks at 7 weeks of age

2002 (October):	reassortant human-like H1N2
2003 (September):	reassortant human-like H1N2
2006 (March):	reassortant human-like H1N? (Ha of human origin)
2006 (August):	reassortant H1N1* (G2)
2006 (September):	avian-like H1N1
2008 (February):	reassortant H1N1 (G2)
2008 (March):	reassortant H1N2 with HA of avian origin ? characterization in progress

* Consequence(s) of the mutation/deletion in antigenic site,
close to the RBS ? (detection, affinity to the receptor ?)

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