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New swine H3N2 and pandemic H1N1 2009 influenza virus reassortant in two Canadian animal populations, mink and swine

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Influenza viruses

Orthomyxoviridae

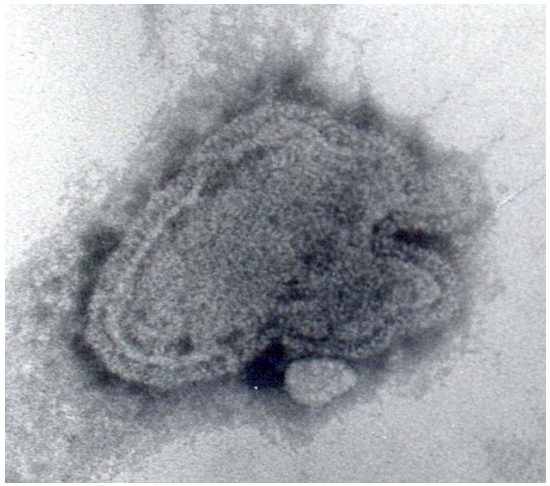
- Most important: Type A Influenza viruses

Segmented genome:

- Negative-sense ssRNA
- A and B Group : 8 segments

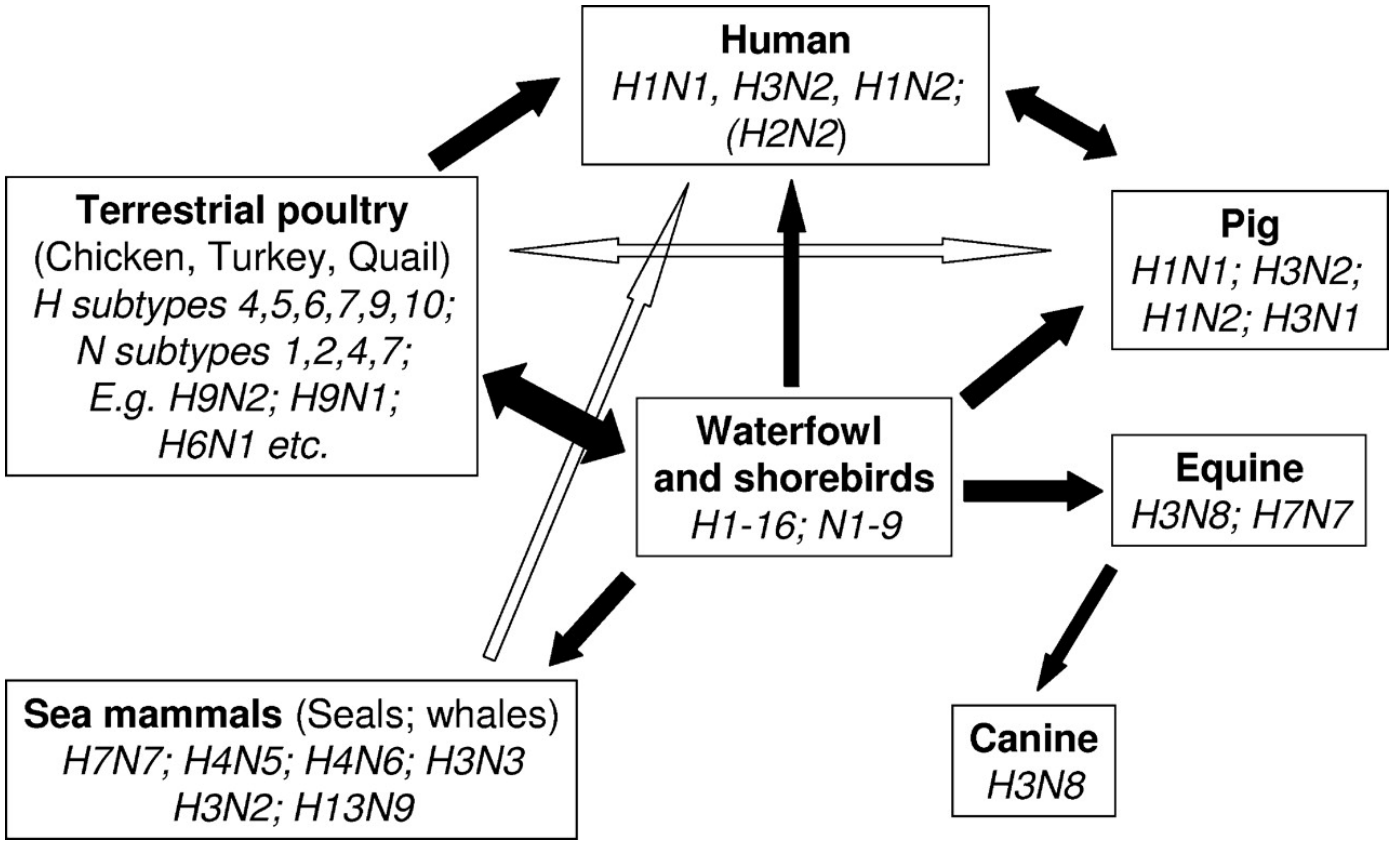
Symptoms

- Fever, coughing, muscles pains, diarrhea, vomiting…



In pigs, diseases caused by influenza viruses do not require declaration

Ecology of influenza A viruses and interspecies transmission



Peiris, J. S. M. et al. 2007. Clin. Microbiol. Rev. 20(2):243-267

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Québec surveillance program

Programme 0222

- Provincial surveillance (MAPAQ)
- Nasal swabs
- Detection and characterization

Came after the avian surveillance (HPAI)

- One health

Inter-species Influenza type A strains

- Genetic mutations

Influenza virus detection

- Matrix (M) gene is targeted
- Reverse Transcriptase PCR (RT-PCR) Real-Time PCR

Characterization of the positive

- Hemagglutinin (H1 et H3)
- Neuraminidase (N1 et N2)
- RT-PCR

H sequencing (or complete genome)

Pandemic strain specific Real-Time PCR

- M gene

2009 H1N1 flu pandemic

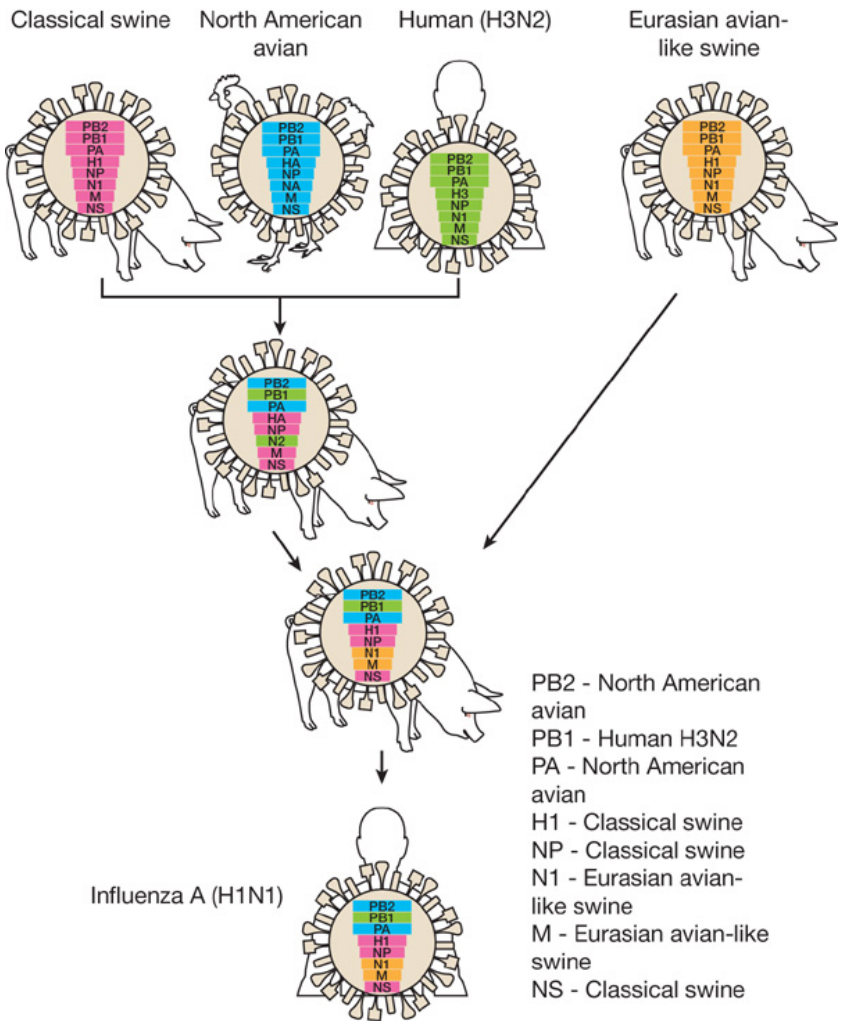
March 18th 2009, Mexican authorities announced 59 death and 854 affected individuals in relation with an influenza-like pneumonia.

- First case declared in Canada: April 2009

- WHO announced on August 2010 the end of the Influenza A/H1N1 pandemic. However, localized outbreaks of various magnitudes are likely to continue.

- Final report of the situation: at least 12 200 deaths around the world.

Genesis of swine-origin H1N1 influenza viruses



G Neumann *et al. Nature* 000, 1-9 (2009) doi:10.1038/nature08157

nature

In December 2009

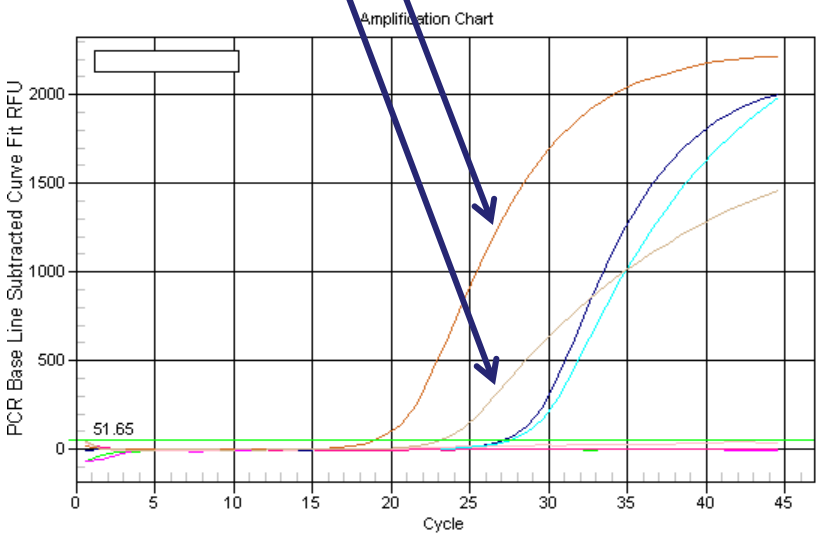
- type A Influenza virus detection on nasal swabs
- Inoculation of embryonated specific eggs and MDCK cell line
- Characterization of the strain: H3N2

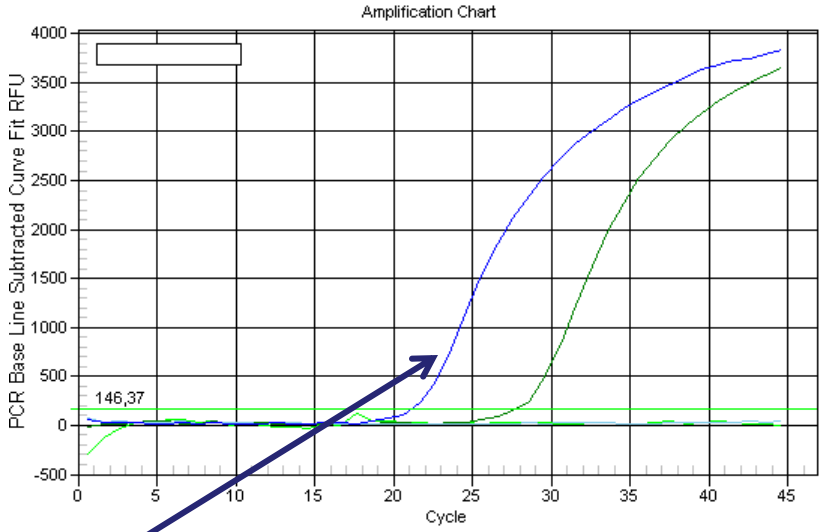
Pandemic M gene!

Type A and H3 positive

Standard Curve Spreadsheet Data

Fluor	Well	Type	Ident.	Rep	Ct	Log SQ	SQ	SQ Mean	SQ SD	Ct Mean	Ct SD	Set Point		
FAM	A01	Unkn	C- FluA	1	N/A	N/A	0.00E+00	0.00E+00		0.00E+00	.00	0.000	N/A	
FAM	A10	Unkn	C- H3	23	N/A	N/A	0.00E+00	0.00E+00		0.00E+00	.00	0.000	N/A	
FAM	B01	Unkn	C+FluA	2	27.05	N/A	0.00E+00	0.00E+00		0.00E+00	27.05		0.000	N/A
FAM	B10	Unkn	C+ H3	24	27.67	N/A	0.00E+00	0.00E+00		0.00E+00	27.67		0.000	N/A
FAM	D02	Unkn	1265553	12	19.03	N/A	0.00E+00	0.00E+00		0.00E+00	19.03		0.000	N/A
FAM	D11	Unkn	1265553	34	23.14	N/A	0.00E+00	0.00E+00		0.00E+00	23.14		0.000	N/A





positive pH1N1

M gene

Standard Curve Spreadsheet Data

Fluor	Well	Type	Ident.	Rep	Ct SQ	Log	SQ Mean	SQ SD	SQ Mean	Ct SD	Ct Point	Set		
FAM	A01	Unkn	C-Swine	1	N/A	N/A	0,00E+00	0,00E+00		0,00E+00	,00	0,000	N/A	
FAM	B01	Unkn	C+Swine	2	27,84	N/A	0,00E+00	0,00E+00		0,00E+00	27,84		0,000	N/A
FAM	D01	Unkn	1265553	4	21,16	N/A	0,00E+00	0,00E+00		0,00E+00	21,16		0,000	

Homologies

Influenza A viral genes segments

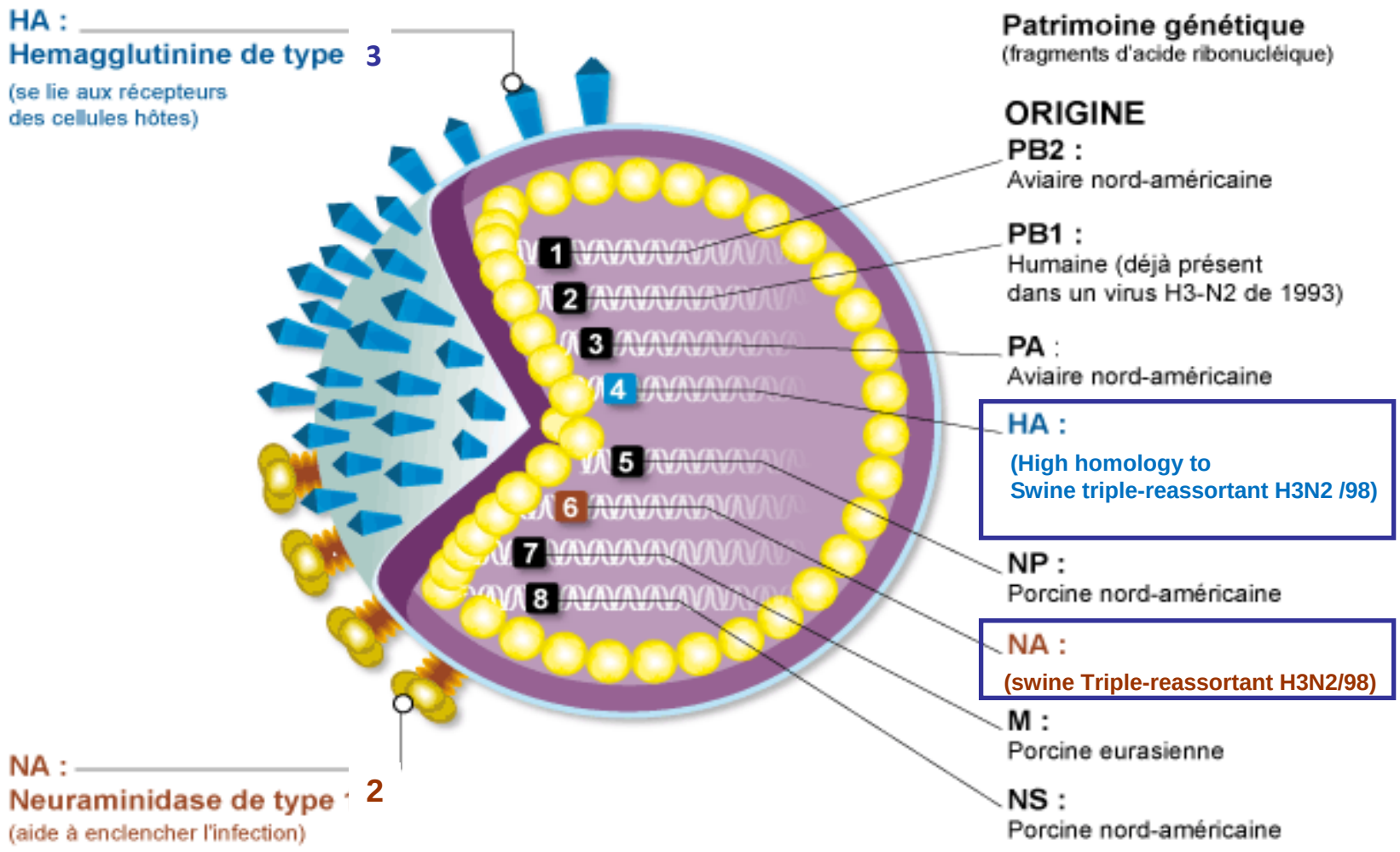
	HA	NA	M	PB1	PB2	PA	NP	NS
pH1N1								
H3N2 SIV cluster IV								
A/swine/Quebec/ 1265553/2010 (H3N2)	95.9%	97.2%	99.5%	99.6%	99.6%	99.1%	99.7%	99.5%

reference strains:

A/NewYork/19/2009 (H1N1)
A/Swine/Québec/4001/2005 (H3N2)

Type A Influenza H3N2/pH1N1 triple reassortant

Ses **huit gènes** proviennent de virus apparus à des époques différentes, dans des zones géographiques variées, qui s'épanouissent chez le porc, les oiseaux et l'homme.



swH3N2/pH1N1

- reshuffling of gene segments has happened between the SIV North-American triple-reassortant H3N2 of 1998 and the pandemic H1N1 2009 strains.
- this reassortant possesses two genes related to SIV H3N2 (HA and NA) and six others related to the pH1N1 (M, PA, PB1, PB2, NP and NS).
- 10% of the piglets were coughing, the sows had no clinical sign and several fattening pigs were coughing with nasal discharge. No increase of death rate was observed.

Retrospective study

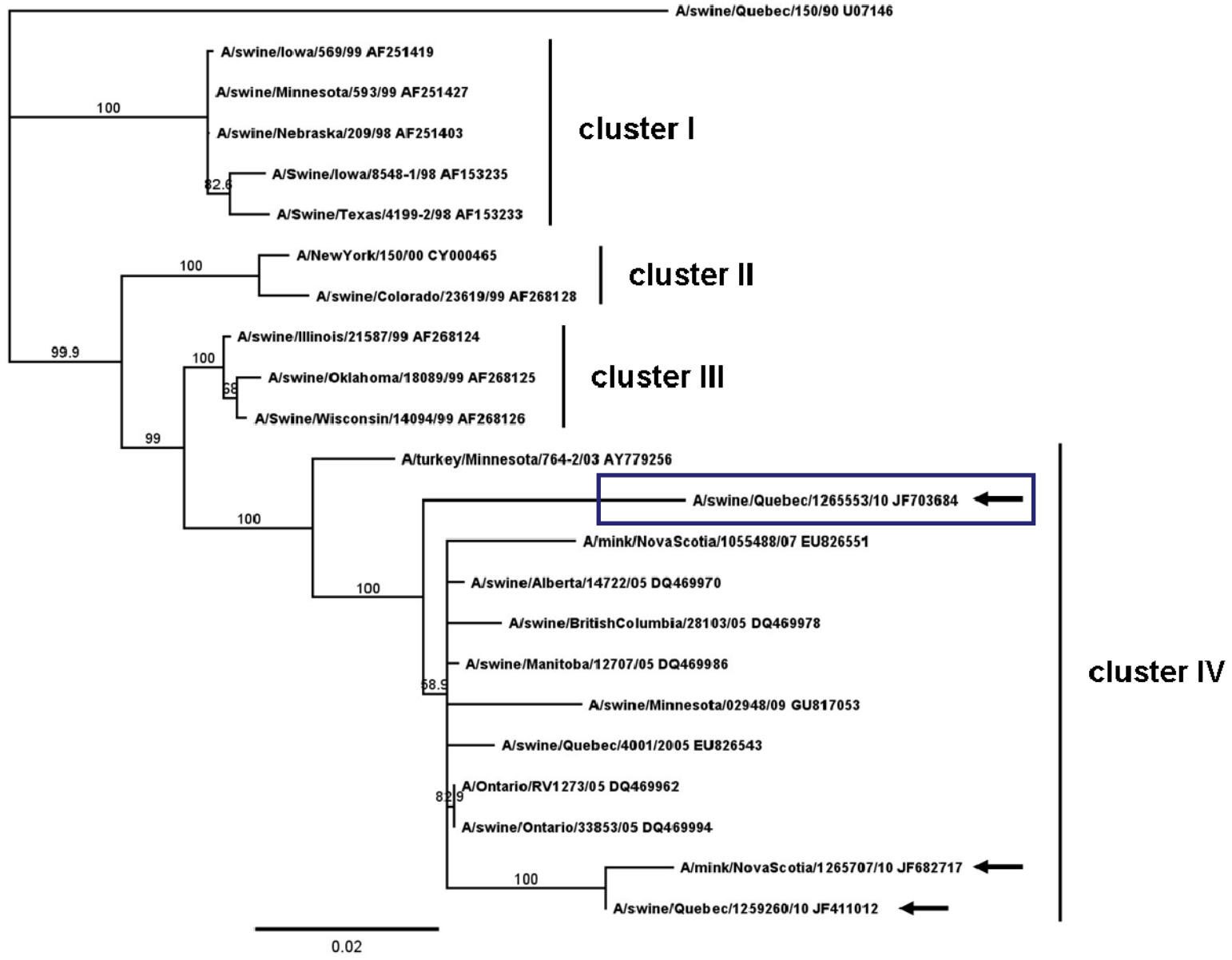
- Octobre 2010 - Janvier 2011
- Different region of Quebec
  - 9/13 H3N2 strains are swH3N2/pH1N1
- Mink tissues
  (Provincial laboratory Nova-Scotia)
  - swH3N2/pH1N1

Homologies

Influenza A viral genes segments

		HA	NA	M	PB1	PB2	PA	NP	NS
Reference strains	pH1N1								
	H3N2 SIV cluster IV								
swH3N2/pH1N1 reassortant strains	A/swine/Quebec/ 1265553/2010 (H3N2)	95.9%	97.2%	99.5%	99.6%	99.6%	99.1%	99.7%	99.5%
	A/mink/Nova Scotia/ 1265707/2010 (H3N2)	95.7% 98,0%	N2 PCR specific	99.2% 99.4%	n.d.	n.d.	n.d.	n.d.	n.d.
H3N2 PCR positive swine diagnostic cases	Nine cases over 13 tested (from October 2010 until February 2011)	H3 PCR specific	N2 PCR specific	97.9% to 100% (partial sequences)					

Phylogenetic analysis



Conclusion

- How and when the pig and mink populations of Canada acquired the new swH3N2/pH1N1 strain is uncertain.
- The fact that the strain was found in a sample recovered from a mink shows the potential of this new virus to cross the species barrier.
- Furthermore, the mink case received from Nova Scotia, indicates a national viral emergence which probably started earlier in 2010.

Conclusion

- M detection and H/N characterization does not differentiate between H3N2 reassortant.
- the new swH3N2/pH1N1 virus has failed to be noticed through the regular surveillance of influenza viruses.
- It is likely that more cases are expected to be positive as surveillance programs adapt their testing.
- We recommend regular sequencing (on selected cases) of the entire Influenza genome as part of a surveillance program.
  - "adequate programmes need to be put in place for surveillance of SIV in the Canadian swine population"

    C. Nfon *et al.* Transboundary and Emerging Diseases, 58: no. doi: 10.1111/j.1865-1682.2011.01219.x

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Les gens. La découverte. L'innovation.