Influenza A virus infections of mink in Denmark 2009-11 – a follow up report April 2012

Lars E Larsen[1], Solvej Ø. Breum[1], Ramona Trebbien[1]; Karolina Bradstad[2]; Lars Peter Nielsen[2]; Mariann Chriél[1], Trine H Jensen[1], Charlotte K Hjulsager[1], Kurt Handberg[1], Poul H Jørgensen[1], Jakob le Févre Harslund[1], Lena Rangstrup-Christensen[1], Bjarne Pedersen[3], Anne Sofie Hammer[1]

[1]National Veterinary Institute, Technical University of Denmark

[2]Statens Serum Institute, Copenhagen, Denmark

[3]Holstebro Veterinary Clinic, Holstebro, Denmark

Mink production in Denmark

- Organized in a cooperative
  - Danish Fur Breeders Association

    Auction house
- 1,700 mink farms
- 2,7 mill breeders
- 13,5 mill mink skin produced
- 5.5 kits per female
- 9 % barren females
- Exports mainly China > 1.2 Bill. EURO

Mink feed production in Denmark

- 14 producers of fresh mink feed (12 cooperatives)
- Main ingredients: fish, chicken, and wheat – and swine waste (lungs, trachea etc!)
- “Complete declarations”

Vare Nr.	%	Fodermiddel:	Teknisk Behandling
244-0	25,00	FJERKRÆBIPRODUKT FARMFOOD	Kogt/frossen.
086-0	13,31	SVENSK SILD	Fersk/frossen.
950-0	10,27	VAND	Vand
667-0	9,55	HVEDE POPPET 90 % <0,5MM	Varmebehandlet korn
009-0	5,00	LAKSEAFFALD	Fersk/frossen.
245-0	5,00	FJERKRÆBIPRODUKT, TYSK VARMT	Kogt/frossen.
343-4	4,00	Svinemix BHJ	Tryksteriliseret/varm.
628-0	4,00	BYG. POPPET TYNDVALSET	Varmebehandlet korn
001-0	3,00	FISKEAFSKÆR < 3% FEDT	Fersk/frossen.
122-0	3,00	H-Pro2004	Syrekonserveret.
244-1	3,00	FJERKRÆ/Fond 75/25	Kogt/frossen.
521-0	2,50	MAJSGLUTEN	Vegetabilsk protein
247-3	2,00	Kyllingefond, Danfond	Syrekonserveret.
871-0	2,00	SOYAOLIE	Vegetabilsk olie
923-0	1,35	Arbocel, LCH-Agro	Fiberstoffer
202-0	1,00	HÆMOGLOBIN. BLODLEGEMER	Fersk/frossen.
201-0	1,00	BLOD (HELBLOD)	Fersk/frossen
362-1	1,00	BLODMEL, SNP, Danfond	Tørrede animalske proteiner
346-1	1,00	KØDMEL P65 DANFOND	Tørrede animalske proteiner
852-0	0,97	SVINEFEDT MAX 1.5 FFA	Animalsk fedt
275-0	0,90	GELATINEHYDROLISA,GELITAFLEX	Tryksteriliseret/frossen
345-0	0,85	GELKO	Syrekonserveret.
917-0	0,20	DPF Period 4 16/07-30/11	Tilsætningsstoffer
909-0	0,10	SALT	
	100,00		

Vægtprocenterne er de nøjagtige vægtprocenter.
(Nettomængde: Se Månedsfaktura)

Indeholder proteinholdige produkter af dyrevæv. Må ikke anvendes som foder til produktionsdyr.

Indeholder fiskemel –må ikke gives til drøvtyggere.

Foderet er konserveret med etoxyquin og eddikesyre

Kategori 2 produkt –ikke til konsum

Opfodringsfærdigt fuldfoder til pelsdyr garanteres at indeholde:

16,3 % Råprotein 10,4 % Råfedt 57,5 % Vand 1,6 % Transfot 2,7 % Råaske 7,8 % Stivelse 0,3 % Sukker

Tilsætningsstoffer pr kg foder:

4500 I.E A-vitamin 700 I.E. D3-vitamin 80 Mg E-vitamin 2,56 Mg Kobber 0,40 Mg Selen

Beregnet indhold pr. kg. Vitaminer og Mineraler

Vitaminer:

30,01 mg	B1-vitamin	16,76 mg	Niacin
10,02 mg	B2-vitamin	0,25 mg	Biotin vitamin H
6,43 mg	B6-vitamin	130,00 mg	BiocholIN
0,06 mg	B12-vitamin	1,80 mg	Folinsyre
6,53 mg	D-pantotensyre		

Mineraler:

3,70 g	Fosfor (P)	5,02 Mg	Mangan naturlig
1,77 g	Kalium (K)	15,59 mg	Mangan Tilsat
1,18 g	Natrium	31,40 Mg	Zink Tilsat
2,26 mg	Kobber naturligt	15,85 Mg	Zink naturlig
86,22 mg	Jern naturlig	0,14 g	Ford. Met./100kcal
70,00 mg	Jern Tilsat	0,24 g	Ford. Tyr./100kcal
		0,33 g	Ford. Phe./100kcal
		0,28 g	Ford. Iso/100kcal

Beregnet indhold pr. 100 gr. blanding:

Tørstof %	Ford. Råprotein %	Ford. Råfedt %	Ford. kulhydrat %	Oms. Energi kcal /100 gr.	Gram ford. Råprot./ 100 kcal
42,5	13,2	10,0	8,4	190,0	7,0

% O.E. Fra:		FK	
Protein:	31,4	80,3	%
Fedt:	50,1	95,0	%
Kulhydrat:	18,5	63,0	%

Outbreaks 2009 – follow up at the farms

- Influenza A was diagnosed in 25 herds out of 54 sampled between August and December 2009
- Brief results of questionnaire:
  - Clinical signs: sneezing, coughing and blood from nose
  - Often died both mink in a case
  - Ten herds reported the number of dead minks – the mean mortality rate was 1.2% (CI: 0.58-1.92)
  - No effect of medication
  - Clinical sign lasted on average 1-2 months
  - Most – but not all – got feed from the same kitchen

Outbreak 2009 – The virus

- Full length sequencing of all 8 segments (sanger)
- Phylogentic analysis

Will the current human vaccine protect against minkH3N2?

HA epitope differences between the H3N2 2009-10 vaccine component (A/Brisbane/10/2007) and the Danish mink 2009-52-1141 sample

Karoline Bragstad
16.10.2009

NOTE: 46 amino acids in the beginning of the HA1 region of the mink virus is missing in the analysis. (Full-length sequence of the mink virus HA will follow). Therefore the discrepancies might be larger than shown.

Epitope	Discrepancies	Differing Residues				
A	4	135	138	140	145	
B	5	158	186	188	193	194
C	1	50				
D	3	173	203	227		
E	0					
Other	3	112	225	323		

Epitope	P	**Dominant Epitope**	**Pepitope**
A	0.210526	**B**	**0.238095238**
B	0.238095		
C	0.037037		
D	0.073171		
E	0		

Conclusion:
The 2009-10 influenza vaccine will not provide efficient protection against the Danish mink virus. It has been proposed that a minimum of four substitutions in two or more antibody binding sites are required for an epidemically important strain.

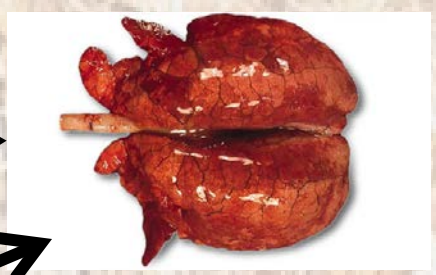
The origin of the mink H3N2 virus

- **HA and NA** genes from **human** seasonal H3N2. Closest to viruses isolated from humans in 2005/2006
- The **M, NP, NS, PA, PB1, PB2** from **swine** H1N1 or H1N2 viruses. Closest to H1N1/H1N2 viruses circulating in Danish swine

- **When and how did it evolved???**

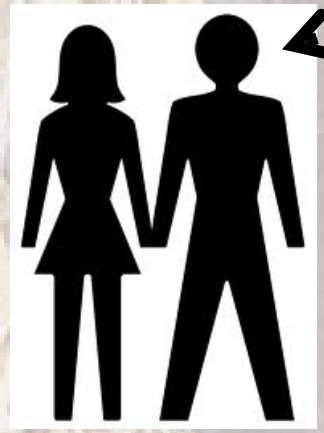
Hypothesis 1: Reassortment happend in swine

Reassortment 2005/6?



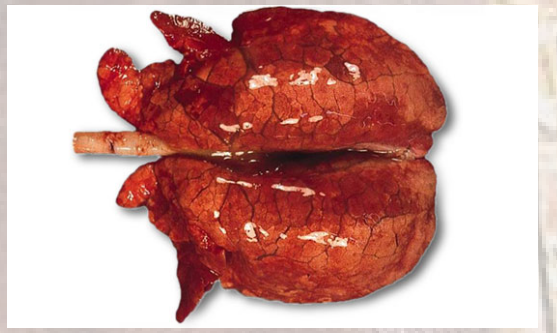
Between 2005 and 2009?

2005/2006?

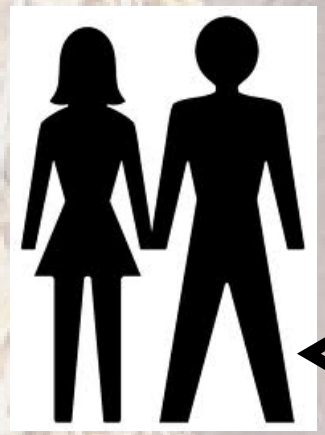


New pandemic?

Hypothesis 2: Reassortment happend in mink



2005/2006?



New pandemic?

Is the mink H3N2 virus circulating?

- In swine?
  - 2008/9: All swine samples found positive in matrix RRT-PCR were tested with a minkH3N2 specific assay with negative results (49 samples).
  - 2010: No test of swine samples
  - 2011: All Influenza positive swine samples (N=90) subtyped by sequencing of N and H. No mink H3N2 identified
- In humans
  - Follow up in 2009 revealed no positives
  - In 2010 and 2011: Not identified but samples are not tested specific for the minkH3N2 and may therefore be missed
- In mink
  - No organized test
    - In 2010: 9 cases tested. 4 positives – two identified as H1N1pdm09; the remaning 2 were not minkH3N2 but were not subtyped further
    - In 2011: 7 cases tested – one positive (H1N1pdm09)
    - In 2012: None tested yet

Conclusions

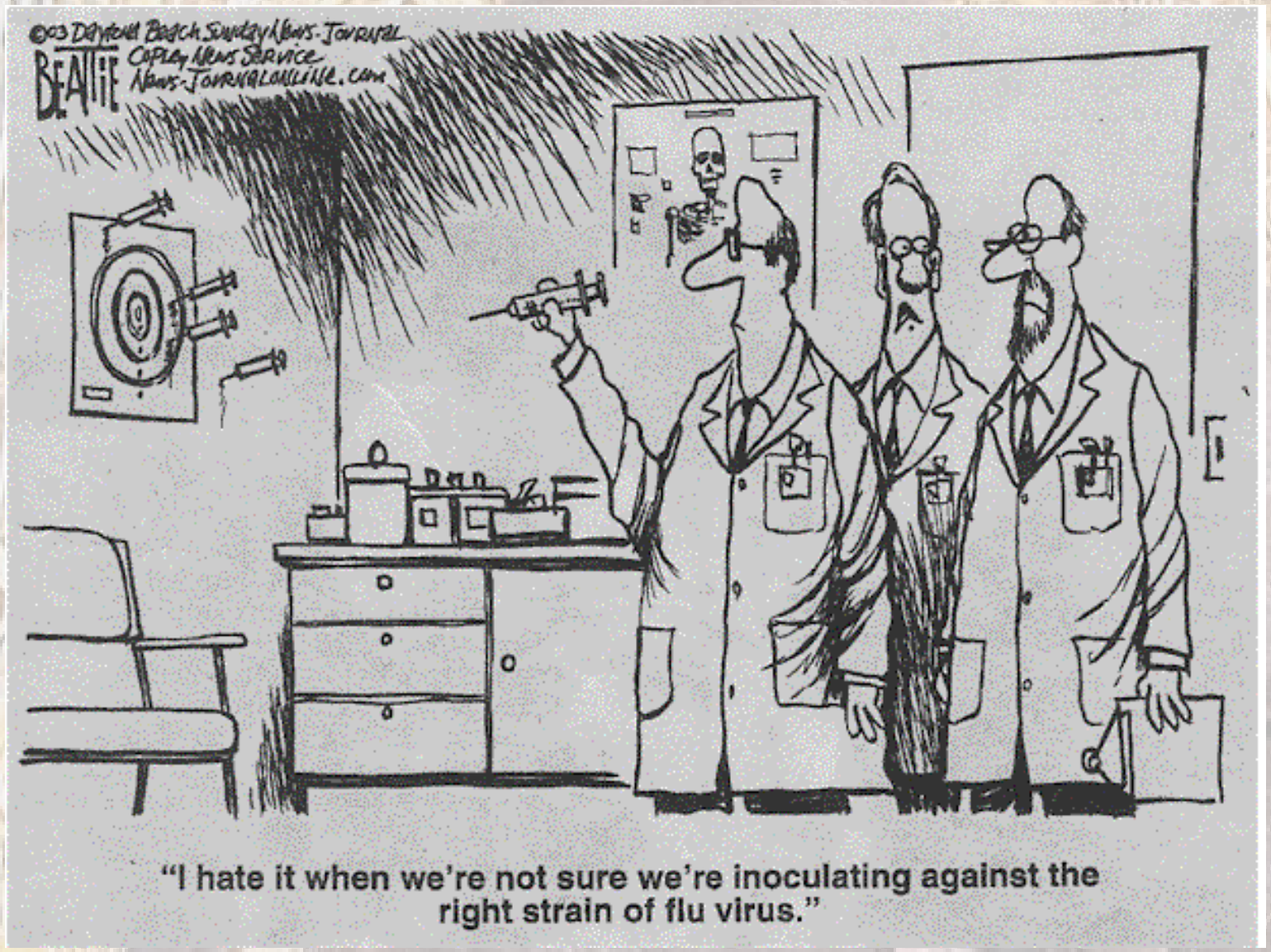
- A new human-swine reassorted virus has been identified
- It is not known if this virus evoked in pigs or in minks, but based on the phylogenetic analysis it may have been circulating since 2005/6
- Introduction probably by feeding uncooked pig tissues but horizontal spread was also seen
- This virus induce severe clinical signs in mink.
- Since the H and N genes are of human origin this virus may be - or may develop into - a zoonotic strain with pandemic potential (current vaccines do not protect)
- There is no indications that this virus is circulating in swine or humans but if the virus is low virulent in pigs the virus may circulate without being noticed due to the lack of active surveillance of influenza A virus in pigs.

Perspectives and further work

- This report and similar recent reports from Canada, USA, Norway and The Netherlands indicate that minks are highly susceptible for influenza A infections.
- Minks are often fed with (uncooked) wast from swine and poultry which may possess a treat for minks acting as mixing vessel. Should this be forbidden?
- Influenza A virus should be considered as a diff. diagnosis of respiratory diseases in mink
- The prevalence of influenza in minks should be tested in all countries with a commercial mink production (China!!)

- Further studies
  - Exp. infection of 8 mink failed
  - Attempt to culture the virus has failed – new cell lines will be tested
  - If the virus can be isolated we want to do exp infection in mink, swine and ferrets
  - Prevalence study of influenza in mink

Thank you!!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"I hate it when we're not sure we're inoculating against the right strain of flu virus."