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Canadian Food Inspection Agency

Our vision:
To excel as a science-based regulator, trusted and respected by Canadians and the international community.

Our mission:
Dedicated to safeguarding food, animals and plants, which enhances the health and well-being of Canada's people, environment and economy.

Molecular and Antigenic Characterisation of Reasortant H3N2 Viruses from Turkeys with a Unique Genetic Constellation

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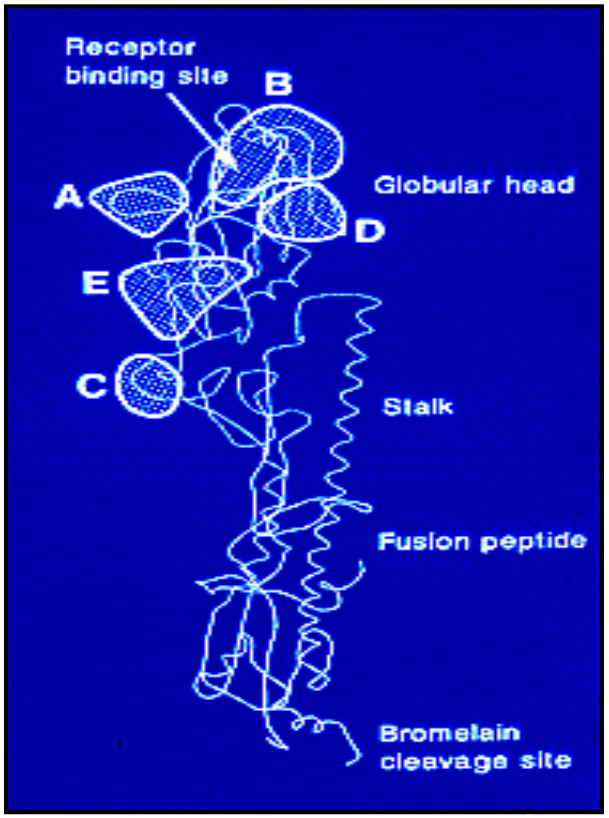
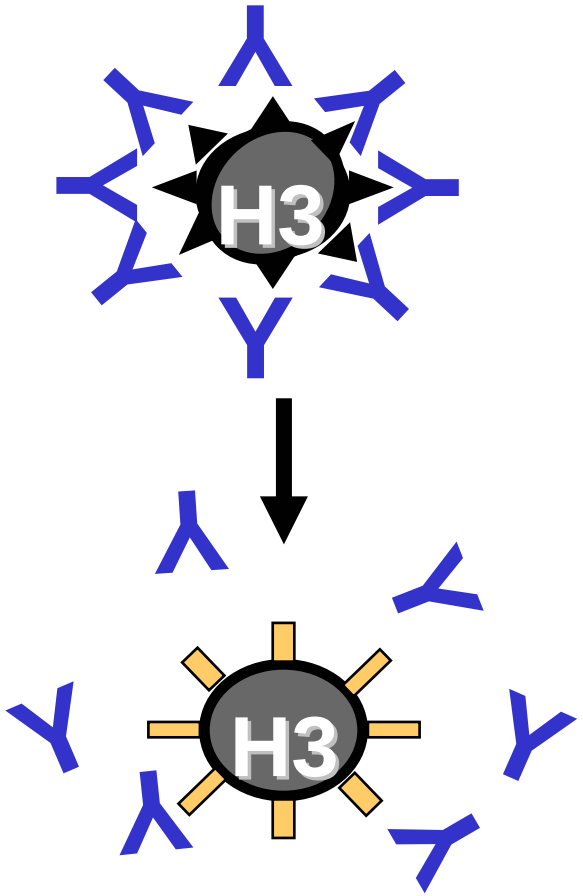
Intro to H3N2 Influenza A viruses

- H3N2 subtype host range: human, swine, avian
- host range restriction primarily due to compatibility of HA protein with host sialic acid receptor
- In swine:
  - endemic worldwide
  - Most recent human-swine transmissions resulted in TRIG H3N2, 2009 pH1N1
  - H3 genotypes to 4 clusters
- In Turkeys:
  - Disease ranges clinically from asymptomatic to severe respiratory tract disease; depression, drop in egg production, and mortality
- In humans:
  - vH3N2 containing 2009 pH1N1 genes found to be transmissible among humans

ANTIGENIC DRIFT: *Immune pressure*
ANTIGENIC SHIFT: *Recombination*





Case Epidemiology

- 3 different farms located in S. Ontario experienced sudden drops in egg production with no other clinic signs
- FAV3: late Feb 15,000 breeder hens
  - molecular subtype H3N2
- Flocks vaccinated with A/mallard/MN/79/79 (H3N4)
- FAV9: early June, 34-week old hens, 10% egg drop
- FAV10: mid June, 20% drop in egg production

Submission Number	Number of swabs	Matrix real time RT-PCR		Isolation in embryonating chicken eggs
		Spackman	Modified	
FAV-003	5	5/5	ND	1/5
FAV-009	6	1/6	6/6	1/6
FAV-0010	7	ND	5/6	3/6

ND = not determined.
doi:10.1371/journal.pone.0032858.t001

Table2. HI titres of pre-exposure sera from H3N4 vaccinated turkeys (FAV10) and convalescent sera from turkeys exposed to TR H3N2 field virus

Serum	A/Tk/BC/01529/2005(H3N2)	A/Mallard/QC/2323-6/2006(H3N2)	A/Tk/ON/FAV003/2011(H3N2)	A/Tk/ON/FAV009/2011(H3N2)	A/Tk/ON/FAV0010/2011(H3N2)
FAV-010 Serum Submission[1]					
1	Neg	Neg	Neg	Neg	Neg
2	16	256	8	16	32
3	Neg	32	Neg	Neg	8
4	16	256	8	8	16
5	Neg	32	Neg	Neg	32
6	32	64	8	16	32
7	Neg	256	8	8	32
8	Neg	256	Neg	8	32
9	Neg	32	Neg	Neg	4
10	Neg	64	Neg	Neg	Neg
11	8	256	8	8	32
12	Neg	256	8	8	32
13	Neg	128	Neg	Neg	32
14	Neg	512	Neg	8	32
15	Neg	1024	8	16	64
16	8	16	Neg	4	32
17	Neg	32	Neg	Neg	8
18	Neg	32	Neg	Neg	16
19	Neg	64	Neg	Neg	16
FAV-003 Serum submission					
20	>8192	64	>8192	256	1024
21	>8192	512	>8192	512	1024
22	>8192	512	>8192	512	4096
23	4096	128	>8192	256	2048
24	2048	64	4096	512	4096
25	>8192	4096	4096	512	2048
26	>8192	4096	4096	1024	4096
27	4096	64	>8192	256	1024
28	>8192	2048	4096	1024	2048
29	>8192	512	1024	512	4096
30	4096	128	>8192	512	2048
31	>8196	256	>8196	512	1024
32	4096	128	4096	512	2048
33	>8192	64	>8192	512	2048
34	>8192	2048	>8192	1024	>8192
35	>8192	128	>8192	512	>8192

[1]Sera from submission FAV-010 tested negative for antibodies to pH1N1 by hemagglutination-inhibition assay.

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Antigenic Characterization

Table3. Characterization of TRH3N2 viruses isolated from turkeys by HI assay using TRBC and various reference antisera

Polyclonal Antisera	Viruses						
	Dk/BC/ 7846/06	Tk/BC/ 1529/05	Dk/ON/ 05/00	Perth/ 16/09	Tk/ON/ FAV003/11	Tk/ON/ FAV009/11	Tk/ON/ FAV010/11
Rabbit 1anti- A/Dk/BC/7846/06 (H3N8)	2048	32	ND	ND	32	16	16
Rabbit 2anti- A/Tk/BC/1529/05 (TR H3N2)	ND	4096	32	ND	2048	128	256
Goat 3anti- A/Dk/ON/05/00 (TR H3N2)	ND	32	2048	ND	32	16	16
Ferret 4anti- A/Perth/16/09 (H3N2)	ND	128	ND	640	512	256	128
Negative Rabbit Serum	0	0	0	0	0	0	0

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- HI assay done using turkey red blood cells
- Polyclonal sera raised against A/Tk/BC1529/05 and A/Perth/16/09 were able to neutralize all 3 newly isolated viruses
- In comparison, A/Dk/ON/05/00 and A/Dk/BC/7849/06 poorly neutralized the viruses

Antigenic Characterization

Table4. Summary of cross neutralization assay results as determined by IPRVN of TR H3N2 viruses isolated from turkeys using various reference antisera

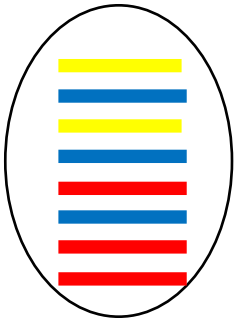
Polyclonal Antisera	Viruses				
	A/Tk/BC/15293/05 (TR H3N2)	A/Dk/BC/7846/06 (H3N8)	A/Tk/ON/FAV003/11 (H3N2)	Tk/ON/FAV009/ 11(H3N2)	A/Mal/QC/2323-66/06 (H3N2)
A/Tk/BC/1529-3/05 (TR H3N2)	2560	ND	640	640	40
A/Dk/BC/7846/06 (H3N8)	80	2560	40	40	2560
A/Perth/16/09 (H3N2)	1280	ND	640	1280	40
FAV-010 (#1) H3N4 vaccinated	40	ND	40	40	1280
FAV-010 (#2) H3N4 vaccinated	40	ND	40	40	2560
Convalescent serum FAV-003 (#1)	2560	ND	2560	2560	1280
Convalescent serum FAV-003 (#2)	1280	ND	1280	1280	2560

ND – Not Done.
doi:10.1371/journal.pone.0032858.t005

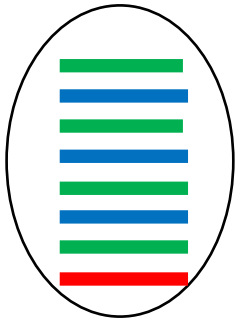
- FAV10 showed strong cross-neutralization activity to avian lineage H3N2 virus but weak activity (1/40 titre) to the recent and earlier TR H3N2 viruses
- Convalescent FAV3 sera cross-neutralized all H3N2 viruses tested (TR, and avian lineage viruses)
    - Indicates potential previous exposure to an H3 virus prior to infection

- NA H3N2 lineage
- TRIG swine lineage
- TRIG avian lineage
- 2009 pH1N1 lineage

FAV3 Isolate

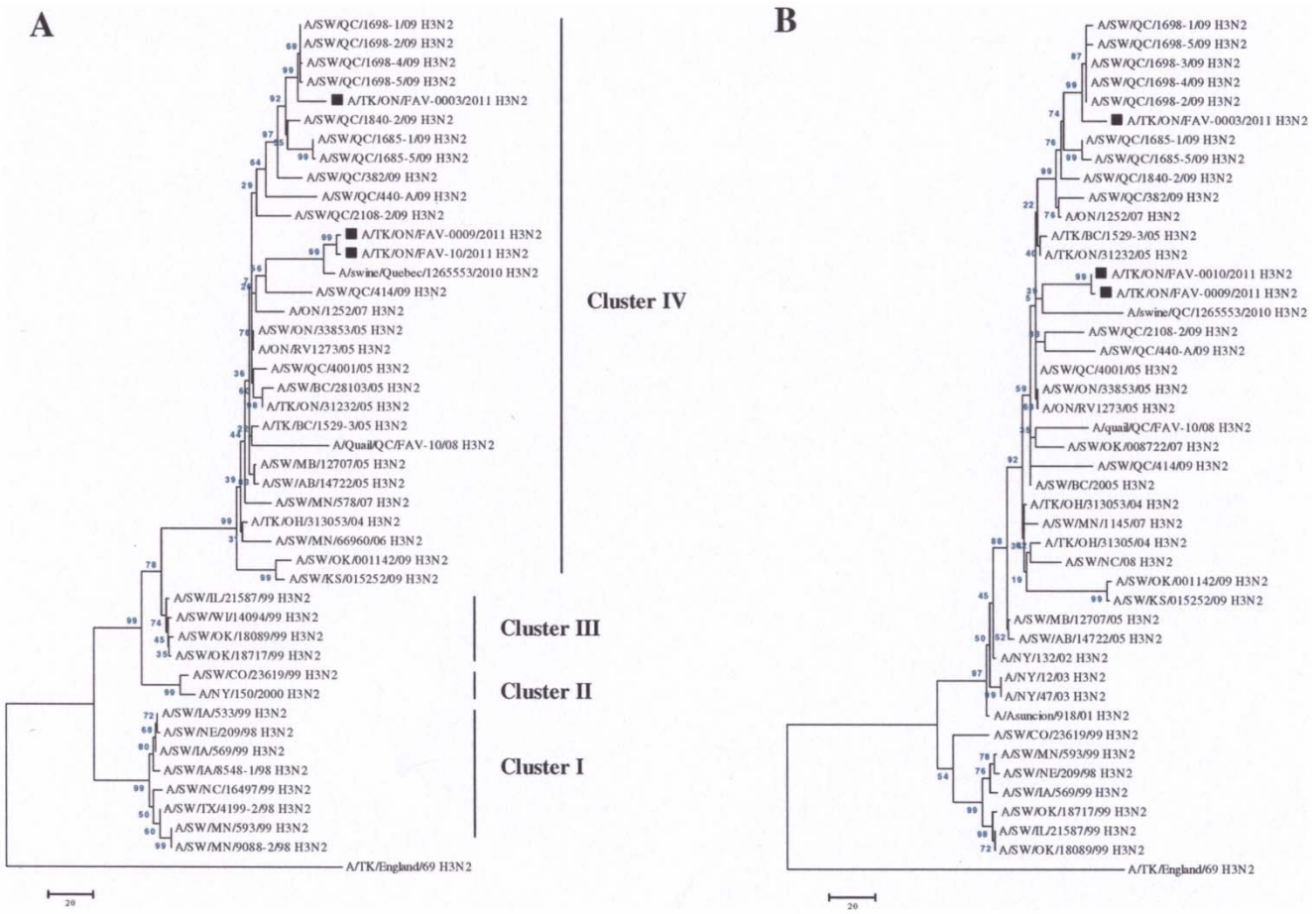


FAV10 Isolate



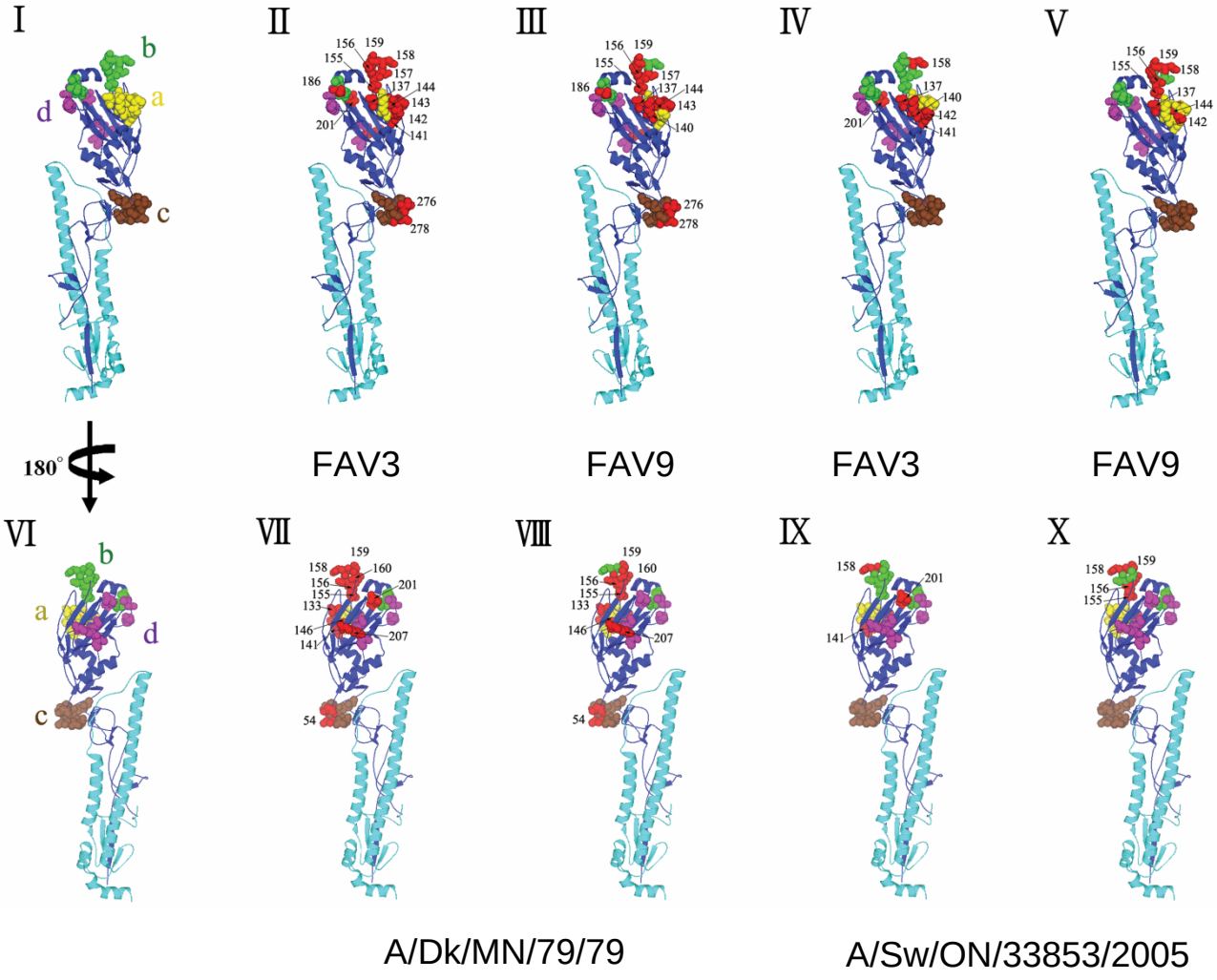
Segment		A/Tk/ON/FAV003/2011(H3N2)	A/Tk/ON/FAV009/2011(H3N2)
PB2	NA	99% A/swine/QC/1840-2/2009(H3N2)	99% A/Ontario/315637/2009(H1N1)
	AA	99% A/swine/QC/1840-2/2009(H3N2)	99% A/Australia/24/2009(H1N1)
PB1	NA	99% A/swine/QC/1698-1/2009(H3N2)	99% A/swine/Minnesota/66853/2006(H3N2)
	AA	99% A/swine/QC/1840-2/2009(H3N2)	99% A/turkey/BC/1529-3/2005(H3N2)
PA	NA	99% A/swine/QC/1698-1/2009(H3N2)	99% A/Ontaio/9739/2009(H1N1)
	AA	99% A/swine/QC/1840-2/2009(H3N2)	99% A/Canada-MB/RV2023/2009(H1N1)
HA	NA	99% A/swine/QC/1698-2/2009(H3N2)	99% A/swine/QC/1268883/2010(H3N2)
	AA	98% A/swine/QC/1840-2/2009(H3N2)	99% A/swine/QC/1268883/2010(H3N2)
NP	NA	99% A/swine/QC/1698-1/2009(H3N2)	99% A/swine/Taiwan/CH-1204/2004(H1N1)
	AA	99% A/swine/QC/1697-1/2009(H3N2)	98% A/Regensburg/D6/2009(H1N1)
NA	NA	99% A/swine/QC/1698-2/2009(H3N2)	98% A/Tk/BC/1529-3/2005(H3N2)
	AA	99% A/swine/QC/1698-5/2009(H3N2)	98% A/Ontario/RV1273/2005(H3N2)
M	NA	99% A/swine/QC/1698-5/2009(H3N2)	99% A/Taiwan/126/2009(H1N1)
	AA	99% A/swine/QC/1840-2/2009(H3N2)	100% A/Ontario/RV1527/2009(H1N1)
NS	NA	98% A/swine/QC/1698-1/2009(H3N2)	98% A/Tk/OH/313053/2004(H3N2)
	AA	97% A/swine/QC/1698-1/2009(H3N2)	97% A/Sw/N.Carolina/02023/2008(H1N1)

doi:10.1371/journal.pone.0032858.t003



Phylogenetic trees of the HA and NA genes created using MEGA4 close-neighbour joining, 500 bootstraps of the maximum parsimony method

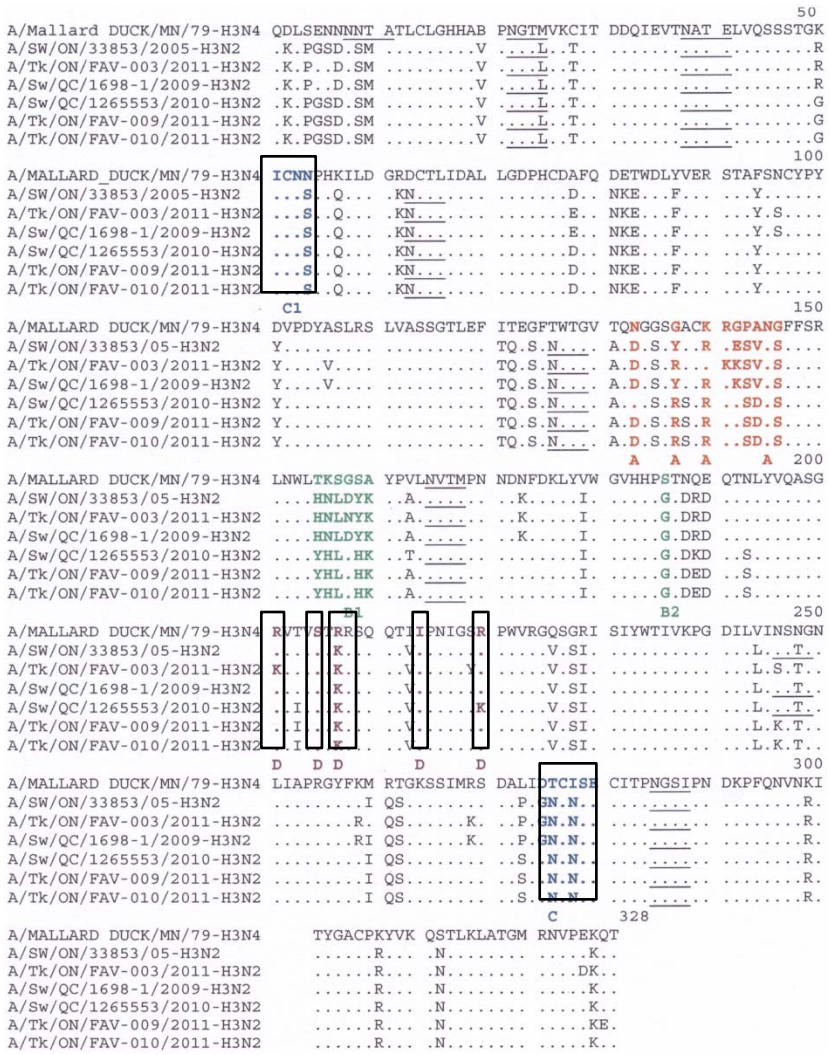
Locations of the AA alterations at the major Antigenic sites of the HA1 Molecule in Crystal Structure



HA1 Alignment

- Pairwise AA alignment of HA1 subunit (signal peptide removed) indicates 79% identity FAV3:vaccine, whereas FAV9/10 had 80% identity
- Predicted AA Identity to prototype cluster IV virus (A/Sw/ON/33853/2005) and all 3 turkey isolates to be 95%
- When compared to one another, the % identity among the turkey isolates decreased to 91%
- Closer inspection of changes at antigenic sites show FAV3 to have 19 AA differences as compared to the vaccine strain, FAV9 to have 16
- FAV3 virus is closest to a swine 2009 TR H3N2 (8AA changes)
- FAV9/10 closest to a 2009 TR H3N2 (5AA changes)
- Predicted N-glycosylation sites determined by NetNGlyc 1.0 indicated good correlation amongst sites, but loss of a potential site at position 246

Alignment of the HA1 Amino Acid Sequences



- Antigenic sites A & B are located near the RBS and often play a key role in escape from neutralizing antibodies
- Substitutions E190D at the RBS has been implicated in loss of ability to bind CRBC
- Key RBS sites

D190, V226, S228: SA α2–6

D190, Q226, G228: SA α2–3

Summary

- First time turkeys have been shown to contain 2009 pH1N1 in combination with TR gene constellations
- Vaccine failure is due to the poor match of the 1979 duck virus at the HA epitopes to currently circulating swine viruses
- Clearly it is time for new vaccines to protect against cross-species transmission in areas of high agricultural mixed-farming
- Continued monitoring of these viruses (TR from swine and turkeys) is needed to assess the impact of antigenic drift at the key at sites A & B

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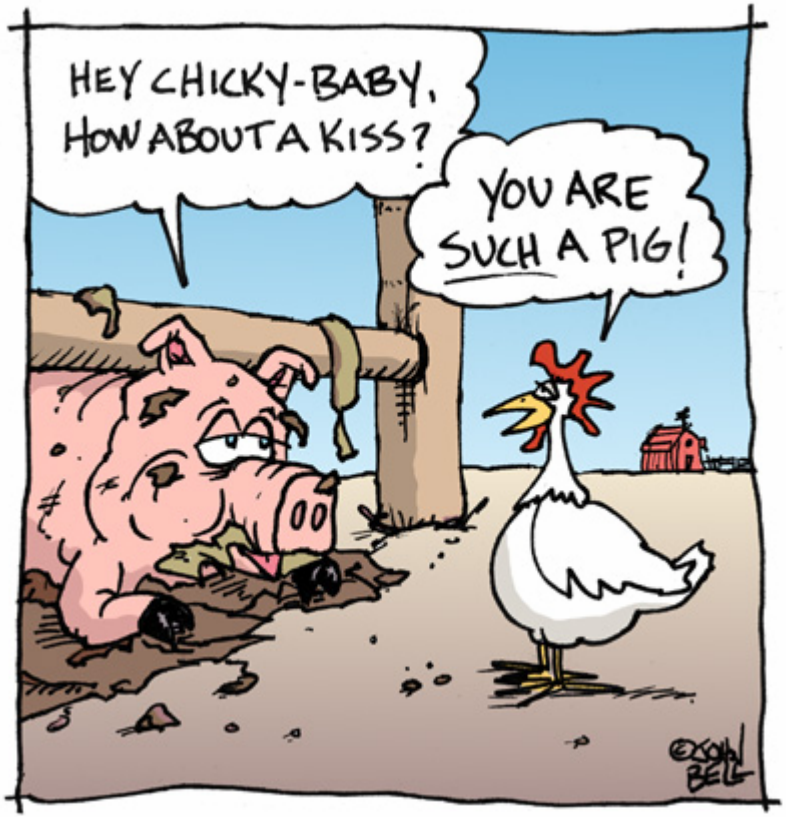

Thank you - Merci

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