



Canadian Food
Inspection Agency

Agence canadienne
d'inspection des aliments



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.

2014 Swine Influenza Update

OFFLU SIV Group Technical Meeting, MN, USA, March 19-20.

Yohannes Berhane, NCFAD, Winnipeg

Canada

Activities related to SIV at NCFAD

- ▶ Summer 2013 started the 2nd phase of SIV surveillance in Canadian swine population
- ▶ Requested SIV samples from Quebec, Manitoba and Ontario for year 2012
- ▶ More than $\frac{3}{4}$ of Canadian swine population located in those 3 provinces
- ▶ We received a total of 93 samples that were positive on SIV matrix based real time RT-PCR



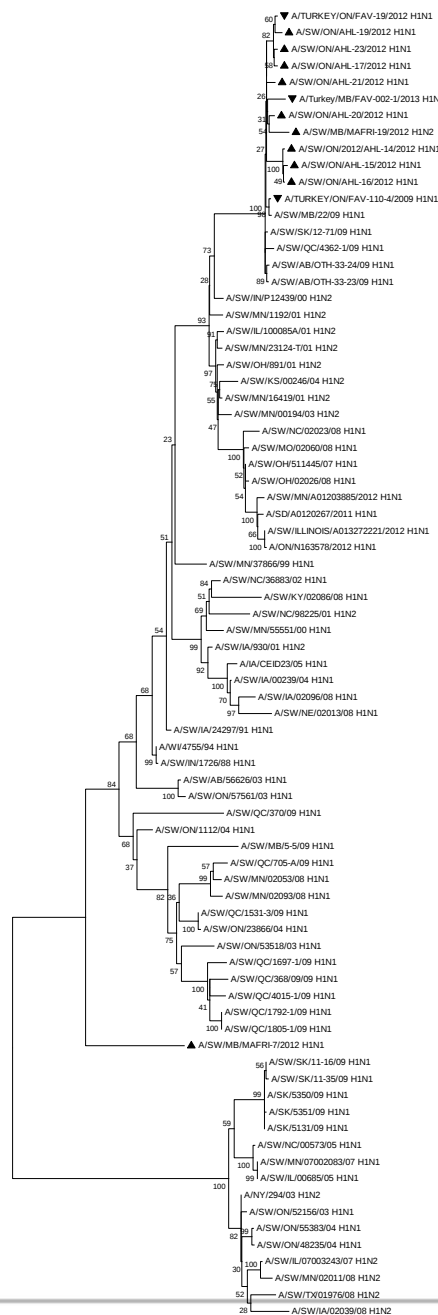
Preliminary Laboratory Results

- ▶ Isolation
 - 67 out of 93 sample yielded isolates
- ▶ Typing using HI assay
 - 31 isolates belong to H1 subtype
 - 20 isolates belong to H3 subtype
 - 16 isolates didn't hemagglutinate

Preliminary Lab results continued...

SIV of H1 subtype from Manitoba and Ontario

Sub. #	PB2	PB1	PA	HA	NP	NA	M	NS
AHL-14	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1
AHL-15	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1
AHL-16	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1
AHL-17	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1
MAFRI-10	TRIG	TRIG	2009 pH1N1	2009 pH1N1	TRIG	N2 TRIG	2009 pH1N1	TRIG
AHL-19	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1
AHL-20	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1
AHL-21	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1
MAFRI-7	TRIG	TRIG	TRIG	cSIV H1	TRIG	N1 Csiv?	TRIG	TRIG
AHL-23	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1	2009 pH1N1



pH1 cluster

γH1 cluster

βH1 cluster

αH1 cluster

δ cluster

Molecular Phylogenetic analysis of the HA genes of SIV isolates of H1 subtype isolated from Manitoba and Ontario by Maximum Likelihood method

Preliminary Lab results continued...

SIV of H1 subtype from Manitoba and Ontario

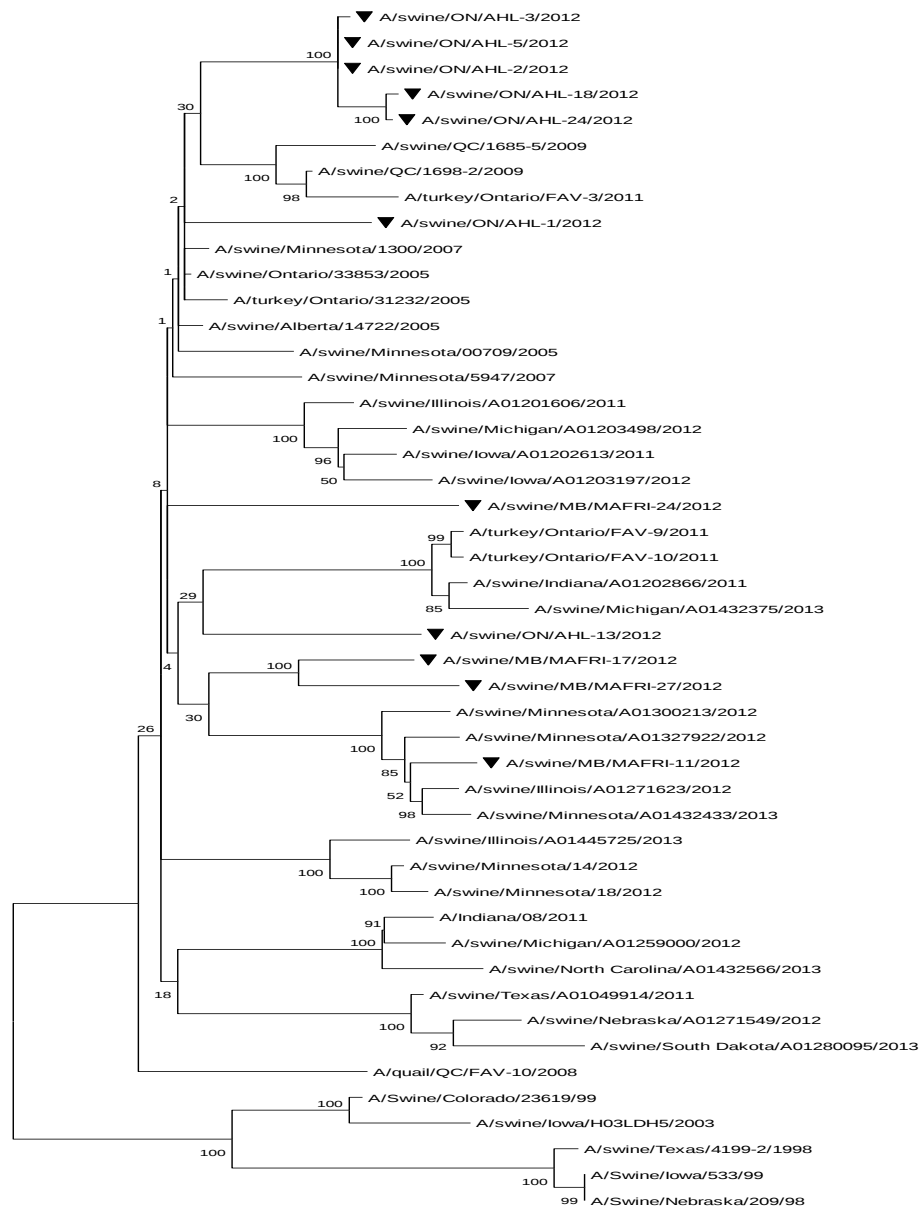
A/Swine/Manitoba/MAFRI-7/2012 (H1N1)

Segment	Amino acid	Nucleotide
HA	91% - A/SW/ON/11112/2004 (H1N1)	90% - A/SW/Iowa/2/1987 (H1N1)
NA	94% - A/SW/Minnesota/01358/2006(H1N1)	94% - A/SW/Minnesota/01358/2006 (H1N1)

Preliminary Lab results continued...

SIV of H3 subtype from Manitoba and Ontario

Sub. #	PB2	PB1	PA	HA	NP	NA	M	NS
MAFRI-1	TRIG	TRIG	TRIG	H3 - TRIG	TRIG	N2 - TRIG	TRIG	TRIG
MAFRI-27	TRIG	TRIG	p2009 H1N1	H3 - TRIG	TRIG	N2 - TRIG	p2009 H1N1	TRIG
MAFRI-17	TRIG	TRIG		H3 - TRIG	TRIG	N2 - cSIV	cSIV	cSIV
MAFRI-24	TRIG		TRIG	H3 - TRIG	p2009 H1N1	N2 - TRIG	TRIG	TRIG
AHL-24	TRIG	TRIG	TRIG	H3 - TRIG	TRIG	N2 - TRIG	TRIG	TRIG
AHL-13	p2009 H1N1	p2009 H1N1	p2009 H1N1	H3 - TRIG	p2009 H1N1	N2 - TRIG	p2009 H1N1	p2009 H1N1
AHL-22	p2009 H1N1	TRIG	p2009 H1N1	H3 - TRIG	p2009 H1N1	N2 - TRIG	p2009 H1N1	p2009 H1N1
AHL-18		TRIG		H3 - TRIG	TRIG	N2 - TRIG	TRIG	TRIG
AHL-5				H3 - TRIG	TRIG	N2 - TRIG	TRIG	TRIG



H3-IV

H3-IV(D)

H3-IV(C)

H3-IV(B)

H3-IV(E)

H3-IV(A)

H3-IV(F)

H3-II

H3-I



SIV in domestic poultry 2011 - 2013

Segment	A/Tk/ON/FAV-3/2011 (H3N2)	A/Tk/ON/FAV-9/2011 (H3N2)	A/Tk/ON/FAV-10/2011 (H3N2)	A/TK/MB/FAV-2/2013 (H1N1)	A/TK/ON/FAV-19/2012 (H1N1)	A/TK/ON/OTH-68/2012 (H1N1)
PB2	TRIG	pH1N1 2009	pH1N1 2009	pH1N1 2009	pH1N1 2009	TRIG
PB1	TRIG	TRIG	TRIG	pH1N1 2009	pH1N1 2009	TRIG
PA	TRIG	pH1N1 2009	pH1N1 2009	pH1N1 2009	pH1N1 2009	TRIG
HA	H3 cluster IV	H3 cluster IV (C)	H3 cluster IV (C)	pH1N1 2009	pH1N1 2009	yH1 cluster
NP	TRIG	pH1N1 2009	pH1N1 2009	pH1N1 2009	pH1N1 2009	TRIG
NA	TRIG	TRIG	TRIG	pH1N1 2009	pH1N1 2009	N1?
M	TRIG	pH1N1 2009	pH1N1 2009	pH1N1 2009	pH1N1 2009	TRIG
NS	TRIG	TRIG	TRIG	pH1N1 2009	pH1N1 2009	TRIG

Future Research work

Finish the full genome sequencing of the remaining SIV isolates for 2012

Next generation sequencing of SIV isolates (Ion Torrent)

Finish Antigenic characterization of the isolated SIV using antiserum panel received from Dr. Amy Vincent (NVSL)

Conduct antigenic cartography in collaboration with Dr. Nicola Lewis (University of Cambridge)

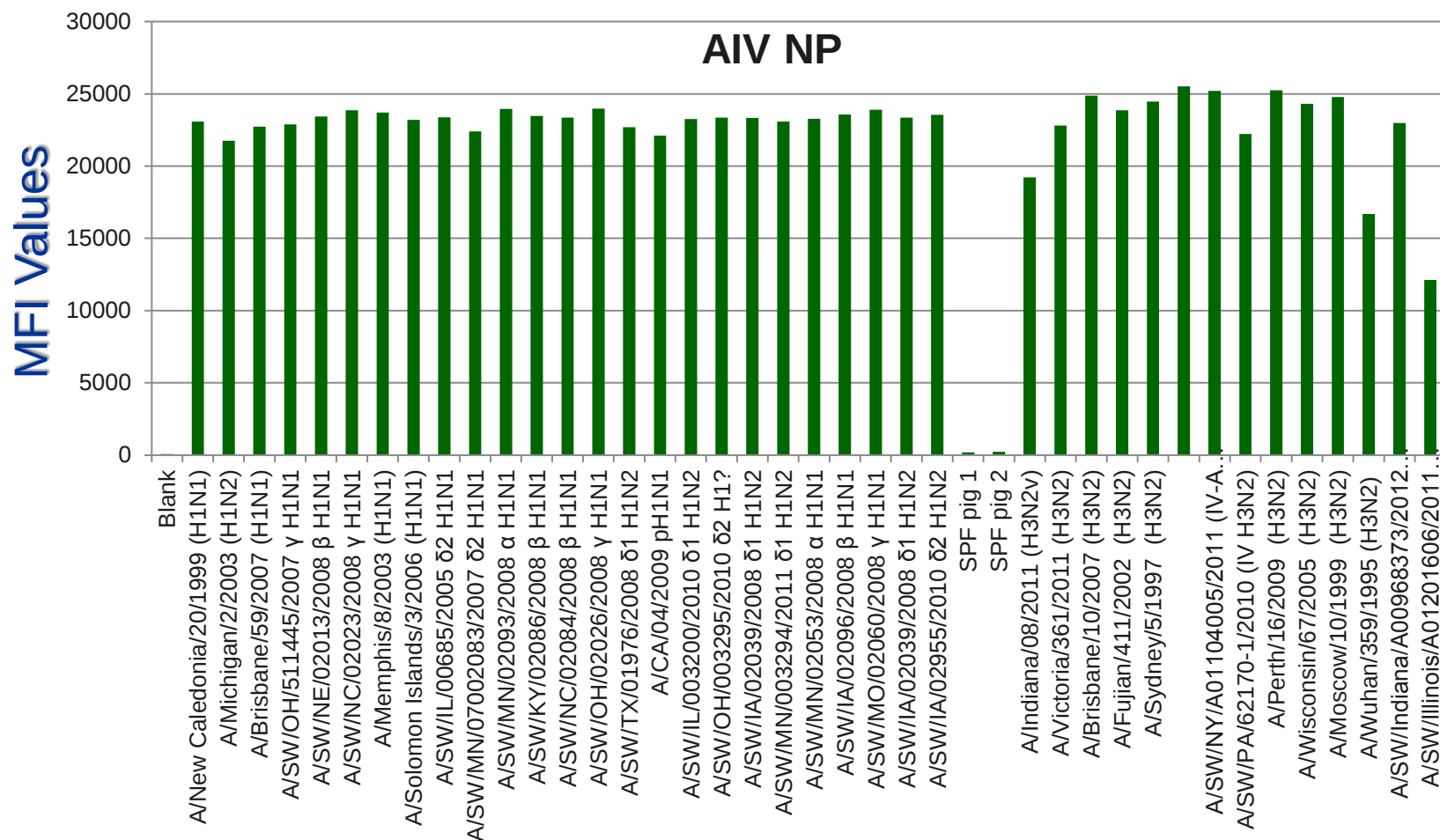
Future Research

- **Swine Innovation Porc – Canadian Swine Research & Development Cluster II**
 - Genetic, Antigenic & Pathobiologic Characterization of Swine Influenza Viruses Isolated from Canadian Pigs
 - Development of a multiplex Luminex immunoassay for serologic diagnosis and subtyping of swine influenza virus (SIV) infections in collaboration with Bio-Vet Inc, Quebec, Canada.

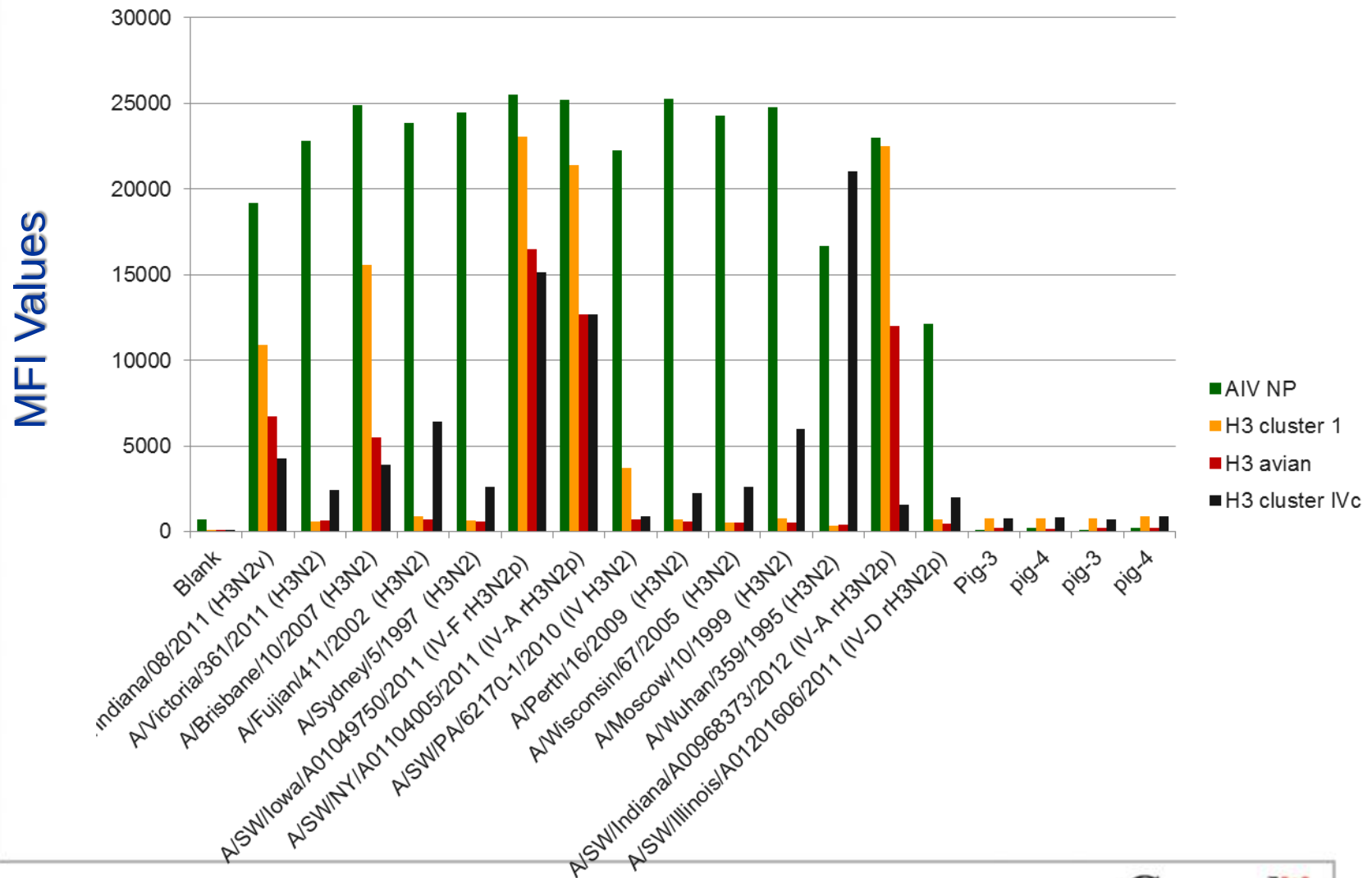
Pilot study – FMIA for SIV

- Development of a multiplex FMIA for the detection of antibodies developed against SIV infection and subtype antibody response to H1 and H3
- Baculovirus expressed AIV nucleoprotein to diagnose SIV infection
- Express the HA1 protein from different clusters of H1 (α , β , δ , γ and pH12009) and H3 (avian, SIV clusters I-IV)
- Select best antigen from each cluster within H1 or H3 subtype which will work better in the assay

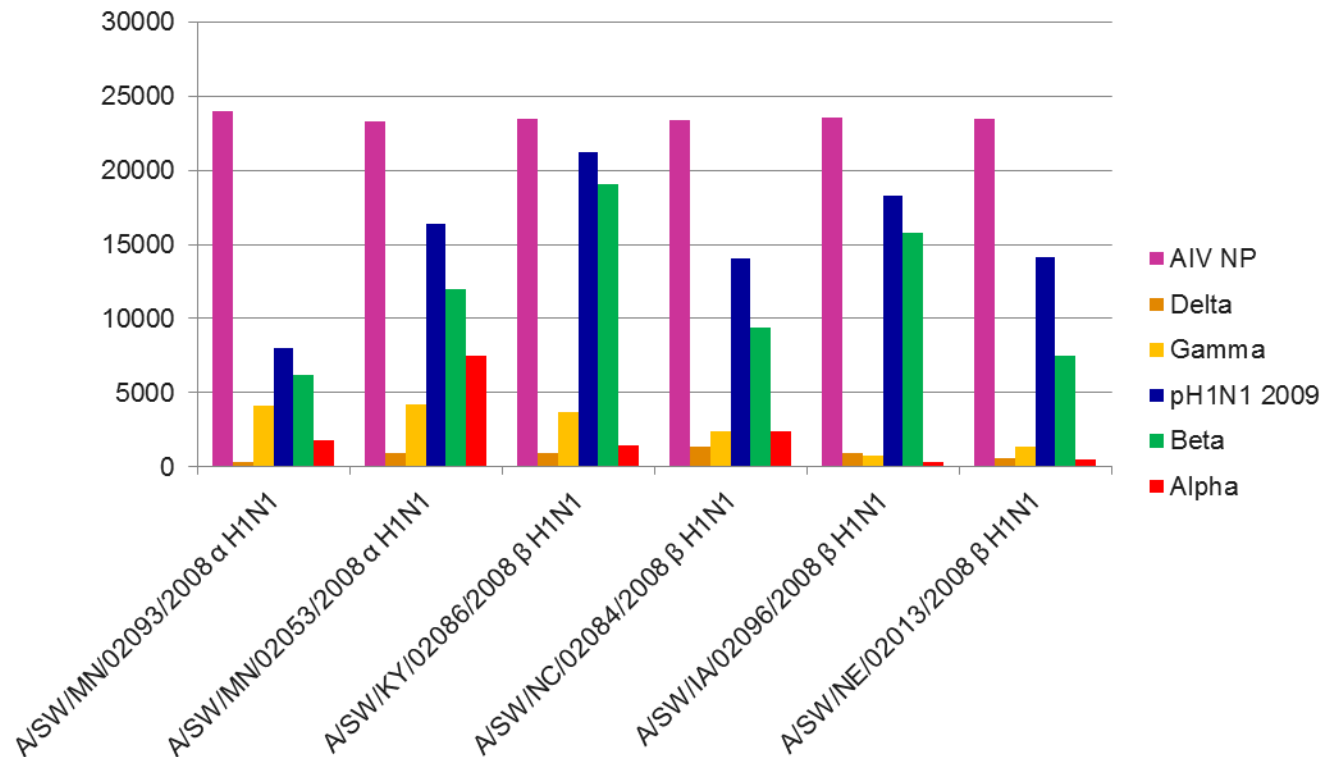
AIV NP based FMIA with panel of SIV reference antisera provided to us by Dr. Amy Vincent



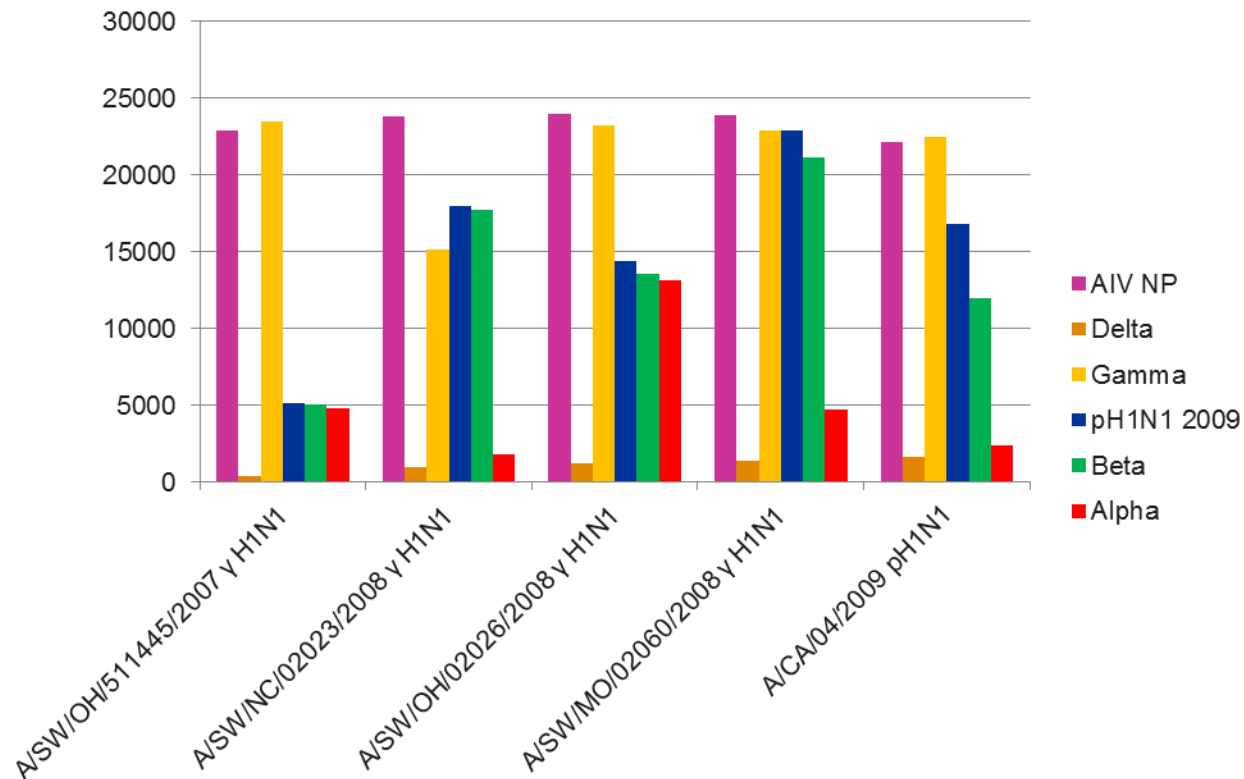
AIV-NP and H3 specific FMIA with panel of H3 SIV reference antisera provided to us by Dr. Amy Vincent



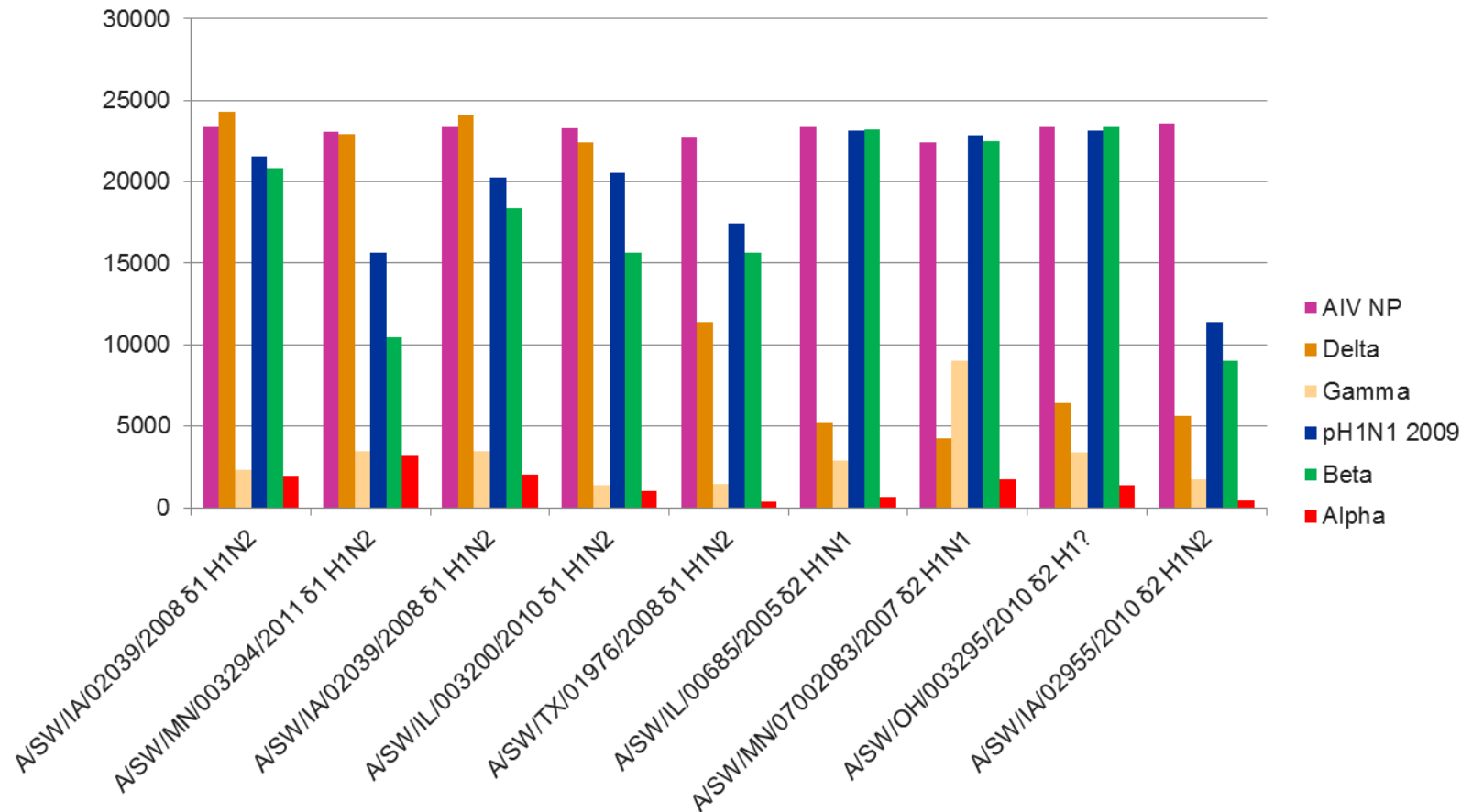
AIV-NP and H1 specific FMIA with panel of SIV reference antisera (H1 α , β) provided to us by Dr. Amy Vincent



AIV-NP and H1 specific FMIA with panel of SIV reference antisera (H1 pH12009, γ) provided to us by Dr. Amy Vincent



AIV-NP and H1 specific FMIA with panel of SIV reference antisera (H1- δ 1, δ 2) provided to us by Dr. Amy Vincent



Future Research on FMIA...

- Express more proteins from other clusters of H1 and H3
- Incorporate internal controls for the assay
- Screen more serum samples from experimentally infected pigs
- Screen more field serum samples from vaccinated pigs



Thank you for your attention