



Medicines & Healthcare products
Regulatory Agency



WP4: NIBSC Ferret studies

Kate Guilfoyle 12th February 2018



WP4 Ferret Studies at NIBSC

- Summary of WIV +/- adjuvant studies
 - FPS: WIV A/PR/8/34
 - FVT: A/Cal/7/2009
 - FPS: WIV A/Cal/7/2009
- Update on DNA Vaccine study
 - FPS: IDAL A/VN/1203/2004



FPS: WIV A/PR/8/34 study outline

- Dose ranging study to establish protective and non-protective WIV doses
- 30 ferret study (5/group)
 - Immunised with: WIV A/PR/8/34 (i.n): Day 0 and Day 21
 - 45µg dose
 - 15µg dose
 - 5µg dose
 - 1µg dose
 - 0.5µg dose
 - PBS
 - Homologous challenge with A/PR/8/1934 (H1N1) (i.n): Day 42
 - Study end: Day 45 (Day 3 post challenge)
 - Tissue samples for virus recovery
(Lung, nasal turbinates, olfactory bulbs & brain)

Results: Tissue sample screening

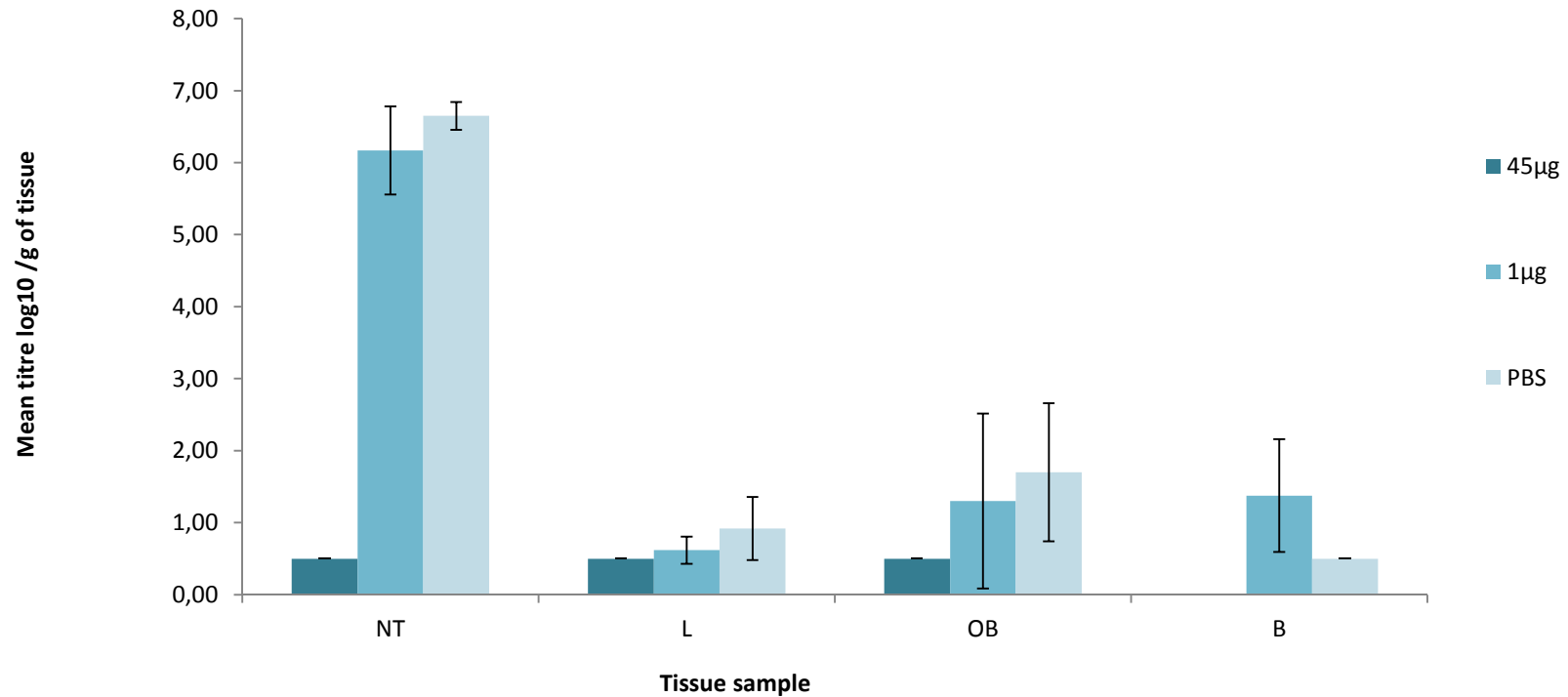
WIV Dose	Ferret Ref	NT	L	OB	B
45µg wIV	F20/17				
	F21/17				
	F32/17				
	F38/17				
	F39/17				
15µg wIV	F22/17				
	F23/17				
	F33/17				
	F40/17				
5µg wIV	F24/17				
	F25/17				
	F34/17				
	F42/17				
1µg wIV	F43/17				
	F26/17				
	F27/17				
	F35/17				
0.5µg	F44/17				
	F45/17				
	F28/17				
	F29/17				
PBS control	F36/17				
	F46/17				
	F47/17				
	F30/17				
	F31/17				
	F37/17				
	F48/17				
	F49/17				

KEY:
 positive
 negative
 NT: nasal turbinates
 L: lungs
 OB: Olfactory bulbs
 B: Brain

- Samples screened on MDCK cells
- Results
 - 45µg dose, no positive samples
 - 15µg and 5µg doses had mixture of positive and negative
 - 1µg dose consistent readout except lung but low positives and not main readout for A/PR/8/34
 - 0.5µg predominantly positive
 - PBS, all positive except for 2 samples from same ferret
- Based on above and further virus recovery assays performed for samples from 45µg, 1µg and PBS groups (see next slide)
 - 45µg chosen as protective dose
 - 1µg chosen as non-protective dose

FPS: WIV A/PR/8/34 Virus recovery results

Mean virus recovery from tissue samples collected Day 3 post challenge with A/PR/8/34 (H1N1)



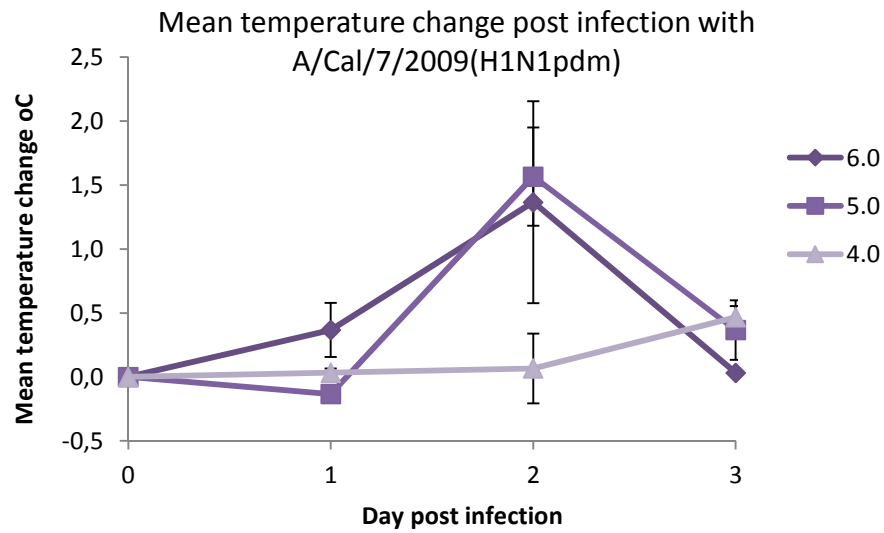
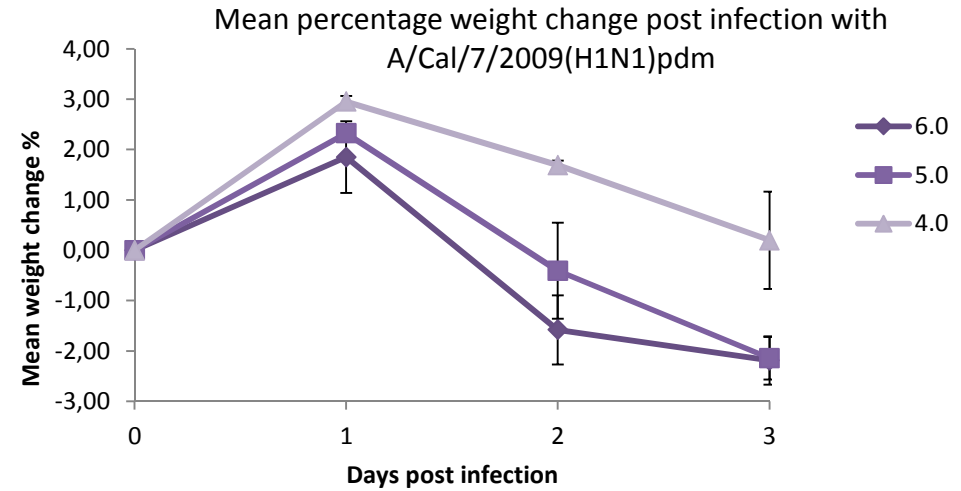
- Geometric mean, limit of detection for assay is 0.5 log₁₀
- n = 5 per group

FVT: A/Cal/7/2009 study outline

- To establish challenge dose to be used
- 9 ferrets (3/group)
 - Infected with: A/California/7/2009 (i.n): Day 0
 - 10^4 EID₅₀/dose
 - 10^5 EID₅₀/dose
 - 10^6 EID₅₀/dose
 - Study end: Day 3
 - Tissue samples for virus recovery
(Lung, nasal turbinates, olfactory bulbs & brain)
 - Weights, temperatures day 0 to day 3



FVT: A/Cal/7/2009



n = 3 per group

Summary:

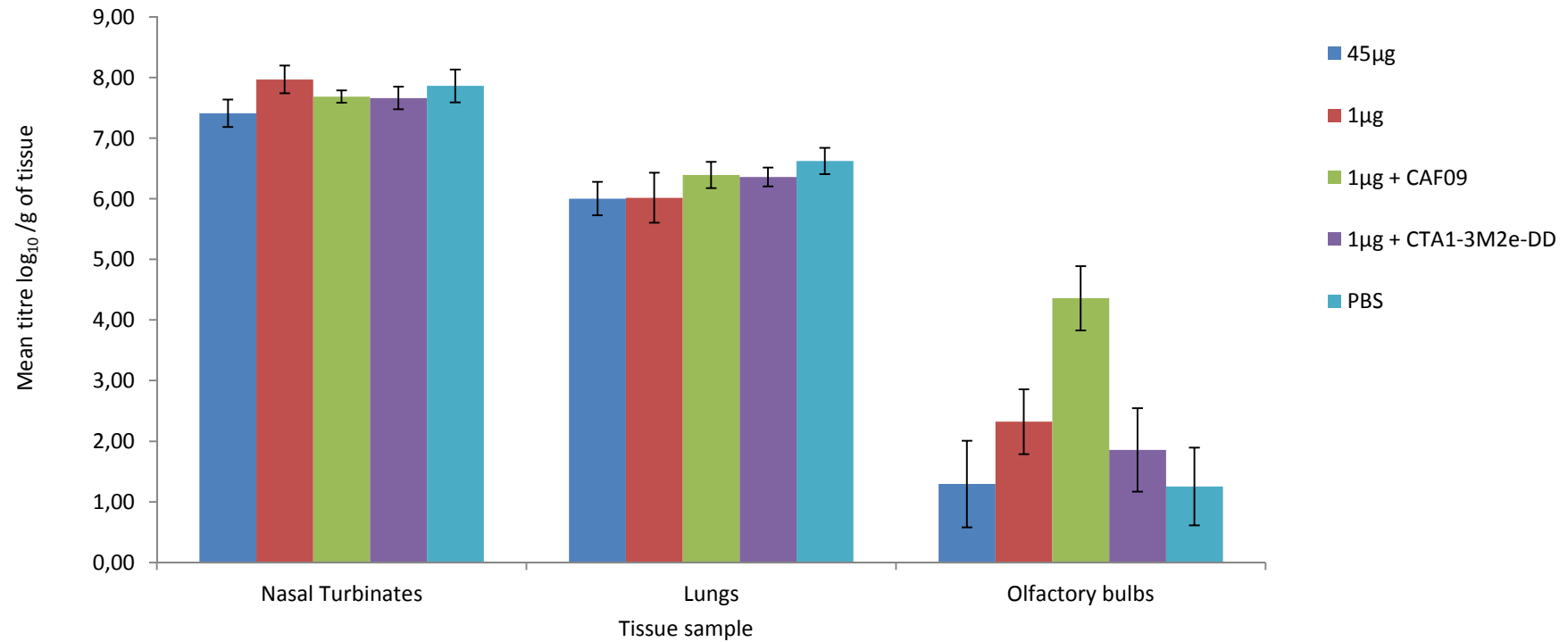
- Established protective and non-protective WIV doses to be used
 - 45µg WIV (protective)
 - 1µg WIV (non-protective)
- Established challenge dose to be used
 - 10^5 EID₅₀/dose

FPS: WIV A/Cal/7/2009 study outline

- 30 ferret study (6/group)
 - Immunised with: WIV A/PR/8/34 (i.n): Day 0 and Day 21
 - 45µg dose WIV (protective)
 - 1µg dose WIV (non-protective)
 - 1µg dose WIV (non-protective) + adjuvant 1 (CAF09)
 - 1µg dose (non-protective) + adjuvant 2 (CTA1-3M2e-DD)
 - PBS
 - Challenged with A/Cal/7/2009 (H1N1pdm) (i.n): Day 42
 - 10^5 EID₅₀/dose
 - Study end: Day 45 (Day 3 post challenge)
 - Gross pathology of lungs
 - Tissue samples for virus recovery (Lungs, nasal turbinates & olfactory bulbs)

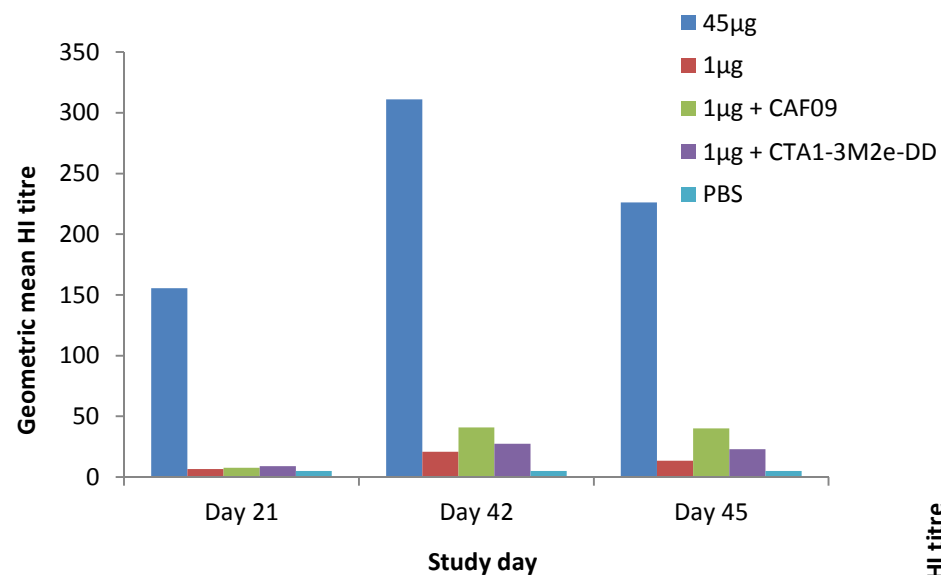
FPS: WIV A/Cal/7/2009 Virus recovery results

Mean virus recovery from tissue samples collected Day 3 post challenge with A/California/7/2009 (H1N1pdm)

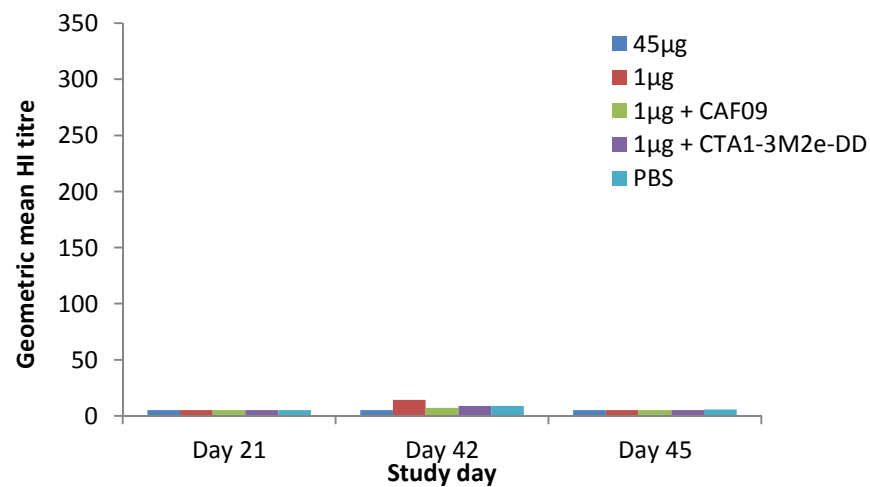


- Geometric mean, limit of detection for assay is 0.5 log₁₀
- n = 6 per group

FPS: WIV A/Cal/7/2009 HI assay results



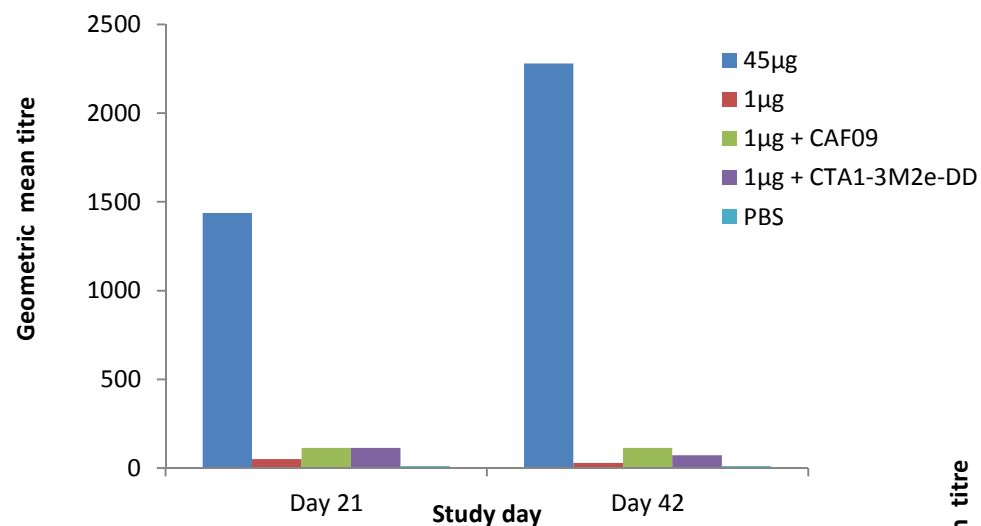
HI assay: A/PR/8/34 virus



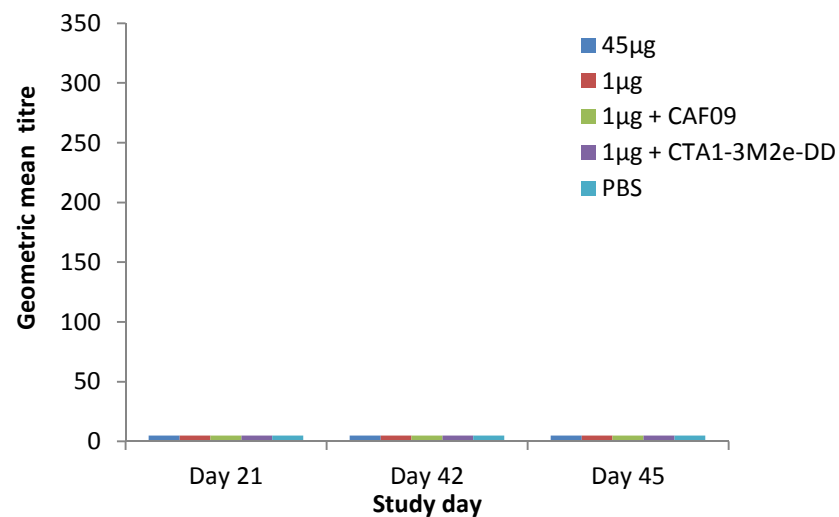
HI assay: A/California/7/2009 virus

Key:
 Day 21 – pre boost
 Day 42 – pre challenge
 Day 45 – post challenge/terminal bleed

FPS: WIV A/Cal/7/2009 VMN assay results



VMN assay: A/PR/8/34 virus



VMN assay: A/California/7/2009 virus

Key:

Day 21 – pre boost

Day 42 – pre challenge

Day 45 – post challenge/terminal bleed

FPS: IDAL A/VN/1203/2004 study outline (1)

- DNA Vaccine:
 - HA & NA H1N1pdm (A/Cal/7/2009)
 - HA & NA H3N1 (A/HK/1/68)
 - M & NP (1918 H1N1)
- 27 ferret study (9/group)
 - Immunised with IDAL gun (t.d): Day 0, Day 14, Day 28
 - 6 Plasmid DNA vaccine
 - 5 Plasmid DNA vaccine (-NP)
 - Diluent only
- Challenge virus A/VietNam/1203/2004 HP H5N1
 - 10^6 EID₅₀/dose

FPS: IDAL A/VN/1203/2004 study outline (2)

- Split into four sets
 - Set 1 and Set 2 challenged (i.n.): Day 38
 - n = 4/immunisation group
 - Sacrificed Day 41 (day 3 post challenge)
 - Gross pathology of lungs
 - Lung samples for histopathology
 - Tissue samples for virus recovery: Lungs, nasal turbinates, olfactory bulbs, brain and spleen
 - Set 3 and Set 4 challenged (i.n.): Day 38
 - n= 5/immunisation group
 - Survival and clinical observations until Day 51 (day 13 post challenge)
 - Weights, temperatures, activity level, food/water intake, etc.
 - Samples taken from euthanised animals during 13 day period post challenge: Lungs, olfactory bulbs, brain and spleen

FPS: IDAL A/VN/1203/2004 Progress

- Set 1 and Set 2 challenged (i.n.): Day 38
 - Sacrificed Day 41 (day 3 post challenge)
 - Gross pathology of lungs
 - Lung samples for histopathology: In progress
 - Tissue samples for virus recovery: Lungs, nasal turbinates, olfactory bulbs, brain and spleen
- Set 3 (n = 2/group) challenged (i.n.): Day 38
 - Survival and clinical observations until Day 51 (day 13 post challenge)
 - Weights, temperatures, activity level, food/water intake, etc.
 - Samples taken from euthanised animals during 13 day period post challenge: Lungs, olfactory bulbs, brain and spleen
 - Gross pathology of lungs
 - Samples for histopathology: In progress
- Set 4 (n = 3/group) challenge(i.n.): Day 38
 - 15th Feb 2018

Thanks to:



- *UNISEC partners*
- *NIBSC colleagues*
 - *BSD*
 - *Influenza group*

