Swine influenza re pandemic H1N1 Key activities at VLA

- Pig infection studies with novel H1N1 virus
  - VLA leading EU funded study
  - Consortium of 10 EU partners
  - Includes use of in-vitro/ex vivo systems
- Scientific evidence base for veterinary and public health risk assessment
  - Development of industry code of practice
  - Trade issues
  - Review surveillance strategies
- Evaluation and modification of diagnostic tools
  - Virus isolation: applicable
  - Virus detection: real time RT-PCR developed and validated
  - Continual data monitoring
  - Seroprofiles including antigenic cartography
- Confirmatory testing facility
  - Singapore Q case

EU Study Consortium

- Nine Institutes & Organisations representing eight EU Member States

- Two affiliates

- Funded by DG SANCO D2 & Defra (UK)

Key Aims

- Provide a robust scientific evidence base for EU Commission & Member States
- Implications of A/H1N1 infection in pigs:
  - Susceptibility of swine: Reverse zoonosis
  - Clinical disease: Herd-level & industry impacts
  - Food Safety: Industry & public health impacts
  - Reservoir: Human & occupational health impacts
- Critical, timely data provision for Veterinary and Public health risk assessments and decision-makers

Study Objectives: A/H1N1 in Pigs

- Infection dynamics
- Host susceptibility
- Clinical outcomes
- Pathogenesis
- Transmissibility
- Experimental infection of pigs with the influenza A (H1N1) virus associated with the global epidemic in humans

Study Design: Overview

- Six groups of 4-5 week old pigs (n=22)
- **Group A:** Direct intra-nasal infection (n=11)
- **Group B:** Control pigs (n=3)
- **Groups C-F:** Contact transmission pigs (n=8)
  - Sequentially introduced in pairs
  - Four cycles of contact transmission at monitored intervals

Study measurements

Sample

- Daily clinical inspection
- Daily swabs: nasal, oral, ocular, rectal
- Post-mortem exam
- Blood samples

Outcome

- Clinical signs
- Shedding profiles: RRT-PCR (& RNA)
- Pathology: Gross, histo, IHC (tissue VI / qPCR)
- Viraemia, serology, APPs, haematology

Results (1)

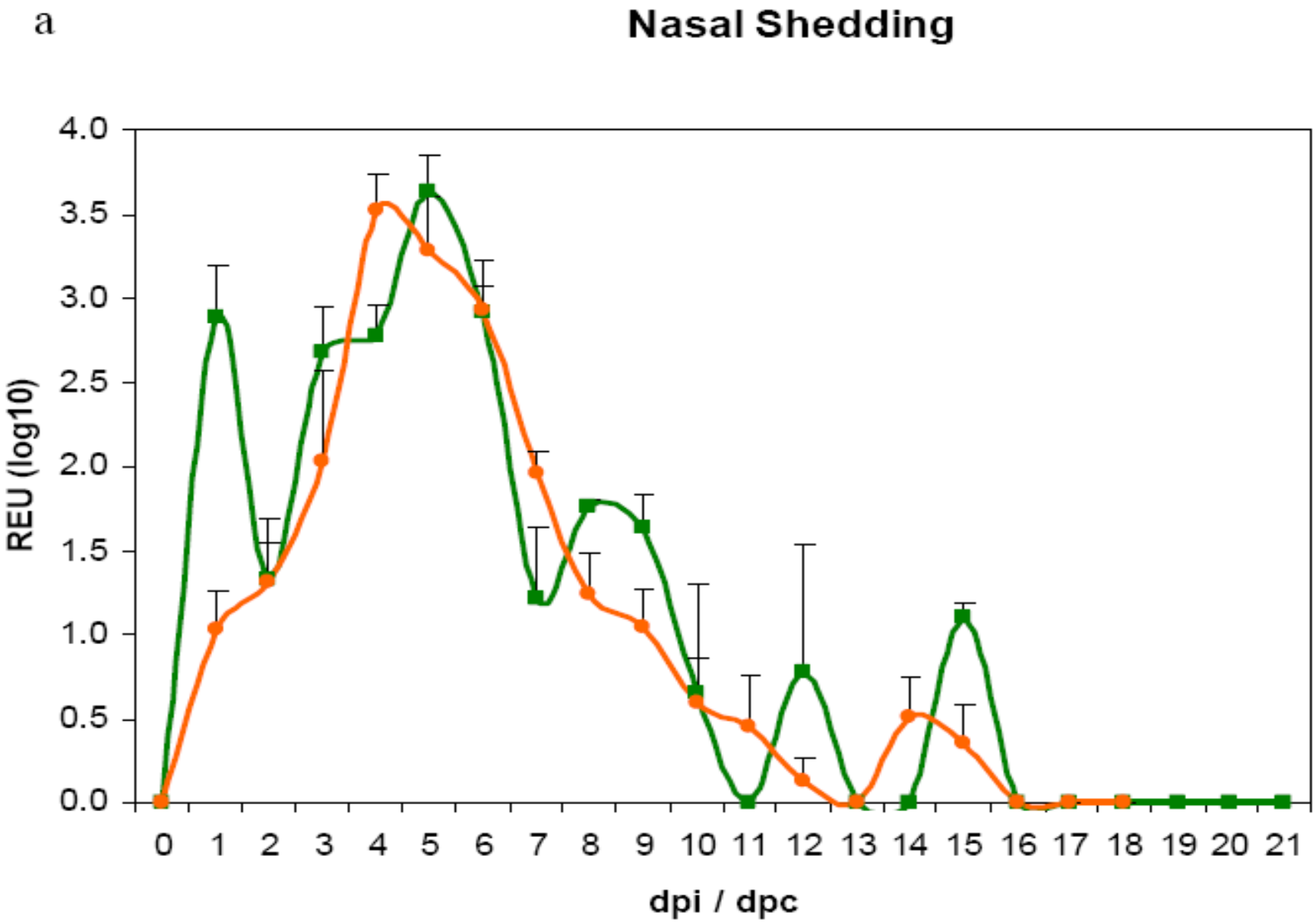
- **Clinical signs**
  - Typical of influenza A infections in pigs
  - Individual variations in range and severity of clinical signs
  - No mortality; low-moderate morbidity
  - Pyrexia (>39.5ºC)
  - Coughing, increased respiratory rate
  - Ocular & nasal discharge
  - Lethargy and inappetence

Results (2)

- **Virus shedding (RRT-PCR)**
  - Nasopharyngeal from dpi 1
  - Peak shedding occurred between dpi 3-5; duration to c16 days
  - Intermittent ocular & oral shedding; no rectal shedding
  - No viral RNA detected in plasma samples dpi 1-7
- **Gross pathology**
  - Dpi 2: Rhinitis; no lung pathology
  - Dpi 3-4: Discrete, focal lobular consolidation
  - Dpi 7: Acute lobular bronchopneumonia

Figure 1 Semi-quantitative H1N1/09 virus shedding from directly infected and transmission cycle pigs

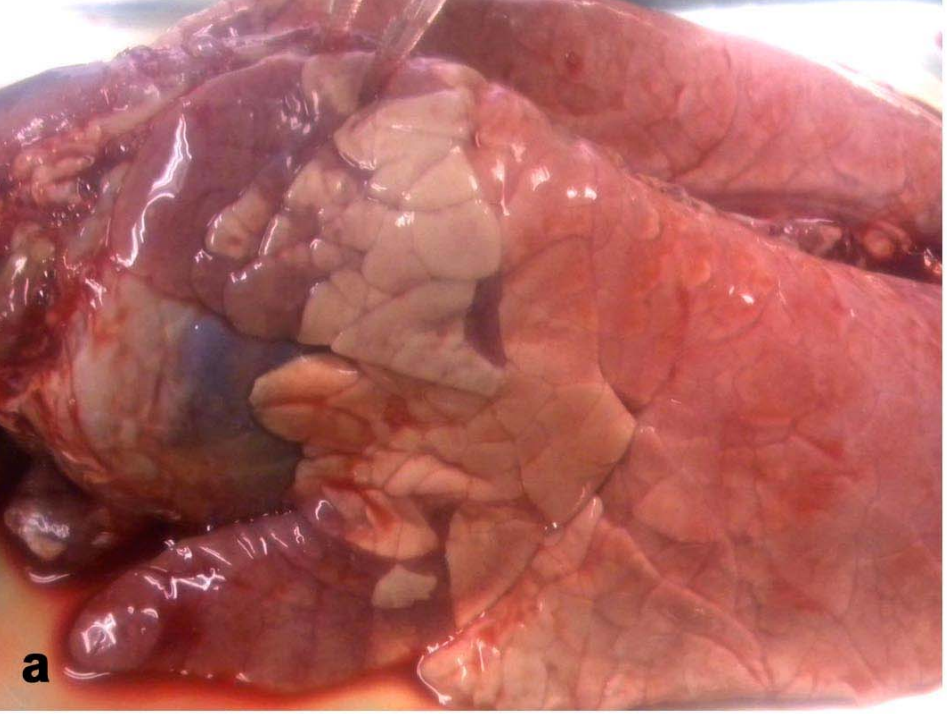
a



Qualitative results for the detection of virus in tissues collected from challenged or contact exposed pigs

Pig ID	PME	Turbinate	Naso-pharynx	Trachea	Middle Lung	Other†
3242	Mock day 3	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-
3249	dpi 2	+/+/+	+/-/+	-/-/-	-/-/-	-/-/-
3252	dpi 2	+/+/+	+/-/+	+/-/+	+/+/+	-/-/-
3246	dpi 3	+/+/+	-/-/+	+/-/-	+/+/+	-/-/-
3243	dpi 4	+/+/+	+/-/+	+/+/+	+/+/+	-/-/-
3260	dpi 7	+/+/+	-/+/-	+/+/+	+/+/-	-/-/-
3244	dpi 7	+/-/+	+/-/-	-/-/-	+/+/-	-/-/-
3257	TC4 dpc 11	-/-/+	-/-/-	-/-/-	-/-/-	-/-/-

Virus re-isolation: immunohistochemistry: PCR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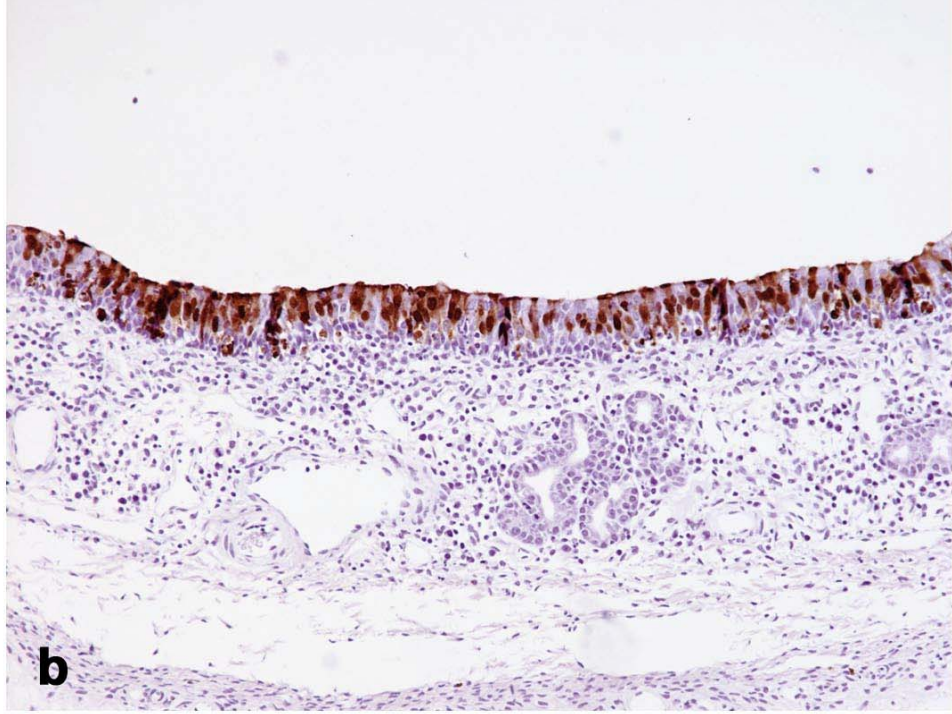
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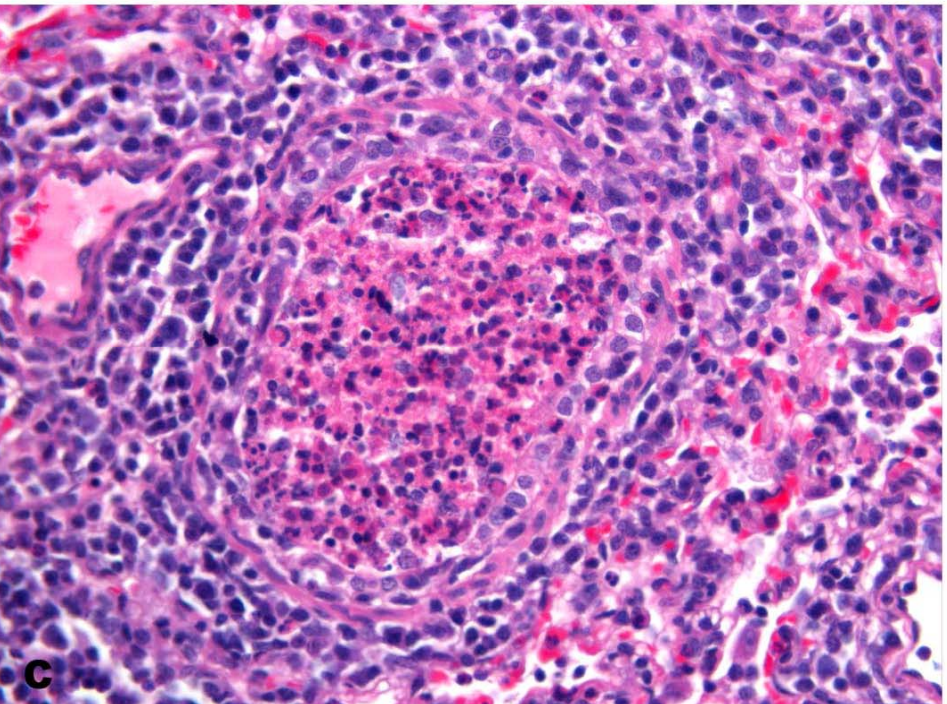
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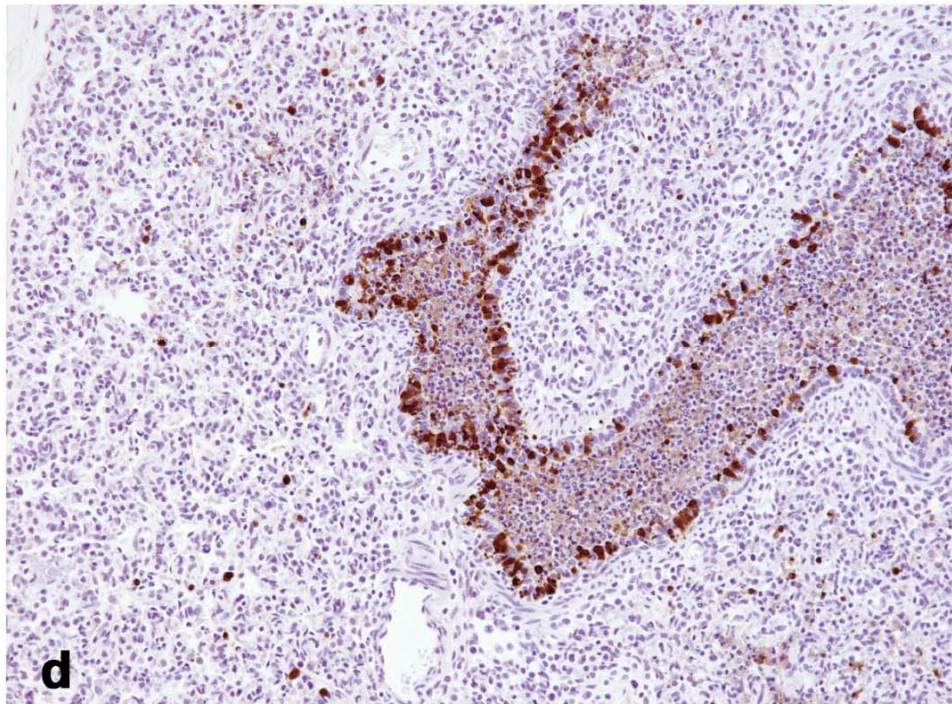
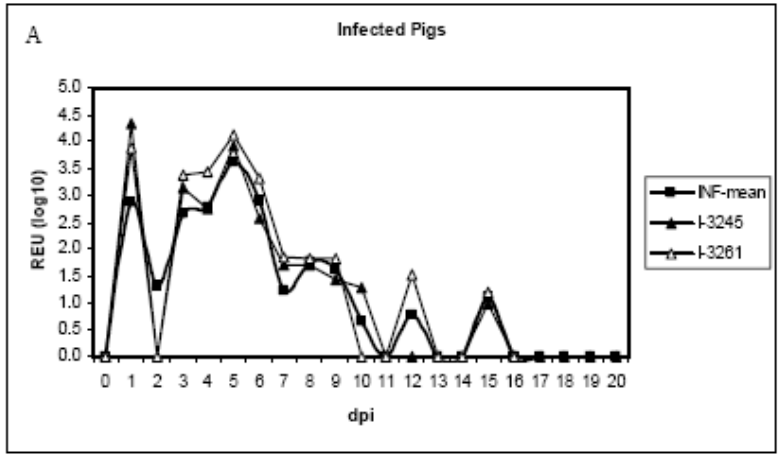
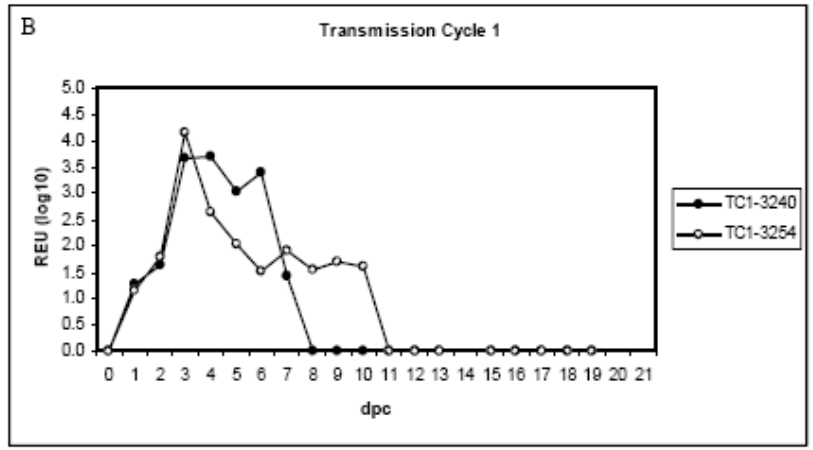
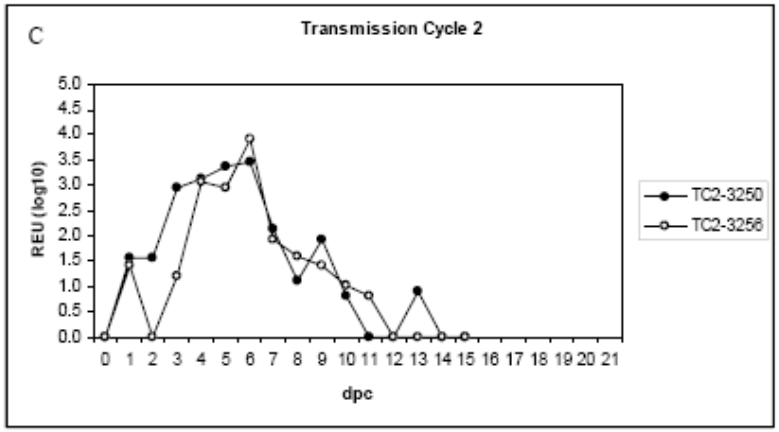

d

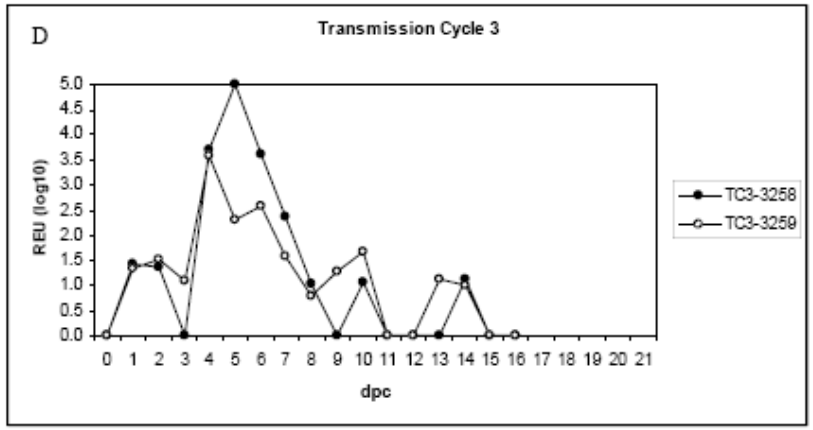
Results: Transmission Cycles - Duration

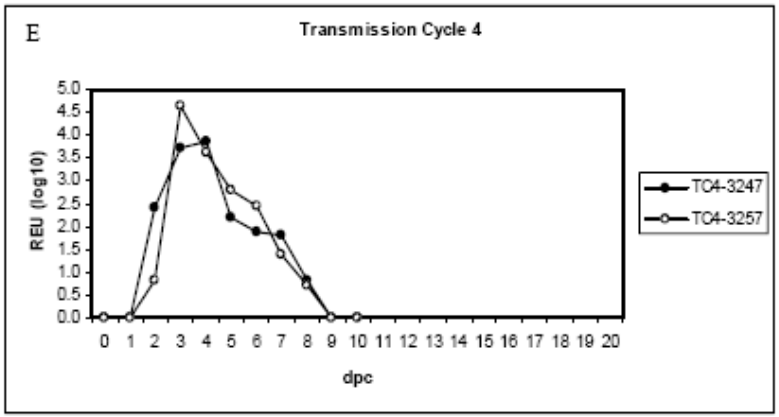
	Infected Pigs nH1N1 (n=11)			Contact Pigs (n=8)					Control pigs (n=3)	
	n=22									
		Room 1	dpi	Clean room	Room 2	Room 3	Room 2*			
Monday	11/05/2009	**Arrive**	-4					11/05/2009		
Tuesday	12/05/2009	*Daily swabbing, weights, temperature*						12/05/2009		
Wednesday	13/05/2009		-2					13/05/2009		
Thursday	14/05/2009		-1					14/05/2009		
Friday	15/05/2009	**Infect I/N (n=11)**	0					15/05/2009		
Saturday	16/05/2009	1x PME-inf	1					16/05/2009		
Sunday	17/05/2009	2x PME-inf	2	**2x Contact (2C) pigs into Room 1**	**72 hours**			17/05/2009		
Monday	18/05/2009	2x PME-inf	3					18/05/2009		
Tuesday	19/05/2009	2x PME-inf	4					19/05/2009		
Wednesday	20/05/2009		5		**2C + 2 naïve (2n) pigs into Room 2**	**72 hours**		20/05/2009		
Thursday	21/05/2009		6					21/05/2009		
Friday	22/05/2009	2x PME-inf	7					22/05/2009		
Saturday	23/05/2009		8			**2n + 2 new naïve (2nn) pigs into Room 3**	**4 days**	23/05/2009		Mock infected pigs (n=2) PME day 3 & day 21
Sunday	24/05/2009		9					24/05/2009	Control pigs (n=1)	
Monday	25/05/2009		10					25/05/2009		
Tuesday	26/05/2009		11					26/05/2009		
Wednesday	27/05/2009		12					27/05/2009		
Thursday	28/05/2009		13					28/05/2009		
Friday	29/05/2009		14				**2nn + 2 pigs (2p) into clean Room 2**	29/05/2009		
Saturday	30/05/2009		15					30/05/2009		
Sunday	31/05/2009		16					31/05/2009		
Monday	01/06/2009		17			***72 hours***		01/06/2009		
Tuesday	02/06/2009		18					02/06/2009		
Wednesday	03/06/2009		19					03/06/2009		
Thursday	04/06/2009		20					04/06/2009		
Friday	05/06/2009		21					05/06/2009		
Saturday	06/06/2009		22					06/06/2009		
Sunday	07/06/2009		23					07/06/2009		

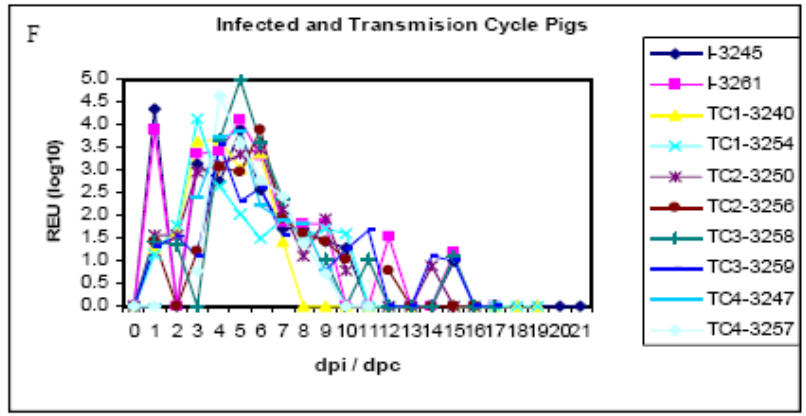

A
Infected Pigs
REU (log10)
dpi
INF-mean
I-3245
I-3261


B
Transmission Cycle 1
REU (log10)
dpc
TC1-3240
TC1-3254


C
Transmission Cycle 2
REU (log10)
dpc
TC2-3250
TC2-3256


D
Transmission Cycle 3
REU (log10)
dpc
TC3-3258
TC3-3259


E
Transmission Cycle 4
REU (log10)
dpc
TC4-3247
TC4-3257


F
Infected and Transmision Cycle Pigs
REU (log10)
dpi / dpc
I-3245
I-3261
TC1-3240
TC1-3254
TC2-3250
TC2-3256
TC3-3258
TC3-3259
TC4-3247
TC4-3257

Immune response

- HI antibody from 7 days
- All pigs beyond 7 dpi/dpc seroconverted

- Acute phase protein response (CRP, Haptoglobin)

A/H1N1 in Pigs: Conclusions

- Pigs are susceptible to infection with A/H1N1 virus
- Induction of detectable levels of:
  - Clinical disease: consistent with endemic SI viruses
  - Virus shedding – 1 to 16 days post infection; peak 3-5 days
  - Pathology
- Variation in severity of disease; no mortality
- Infected animals were able to transmit the virus to naïve contact pigs successively for at least four cycles of transmission
  - Virus selection at genetic level
- Indicates A/H1N1 could become established in susceptible pig populations if introduced
  - Economic impacts
  - Relevance to animal and public health & food safety

Future perspectives

- Further spread to pigs likely
- Endemic infection in swine?
  - Complex dynamic
  - Consistent with historical epidemiology of pandemic viruses
- Other hosts?
- Enhanced surveillance required
- Proportionate disease control responses

Acknowledgements

- Avian Virology & Mammalian influenza group at VLA
  - Sharon Brookes
  - Alex Nunez - Pathology
  - Steve Essen
  - Bhudipa Choudhury
  - Richard Irvine
  - Marek Slomka
  - Anstice Densham
  - Jill Banks
- EU commission (DG Sanco, Defra)
- HPA
- WHO global network
- OIE/FAO laboratory network
- European Surveillance Network for Influenza in Pigs