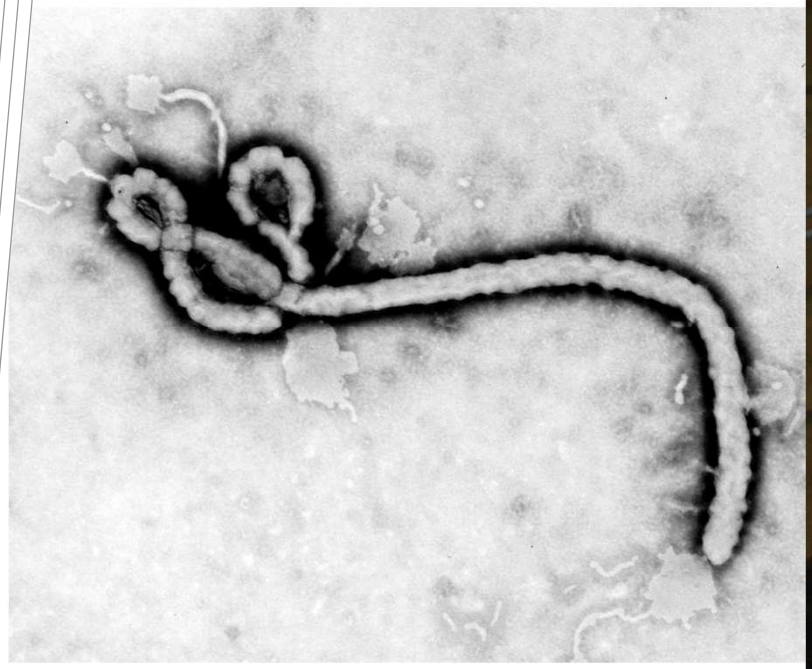

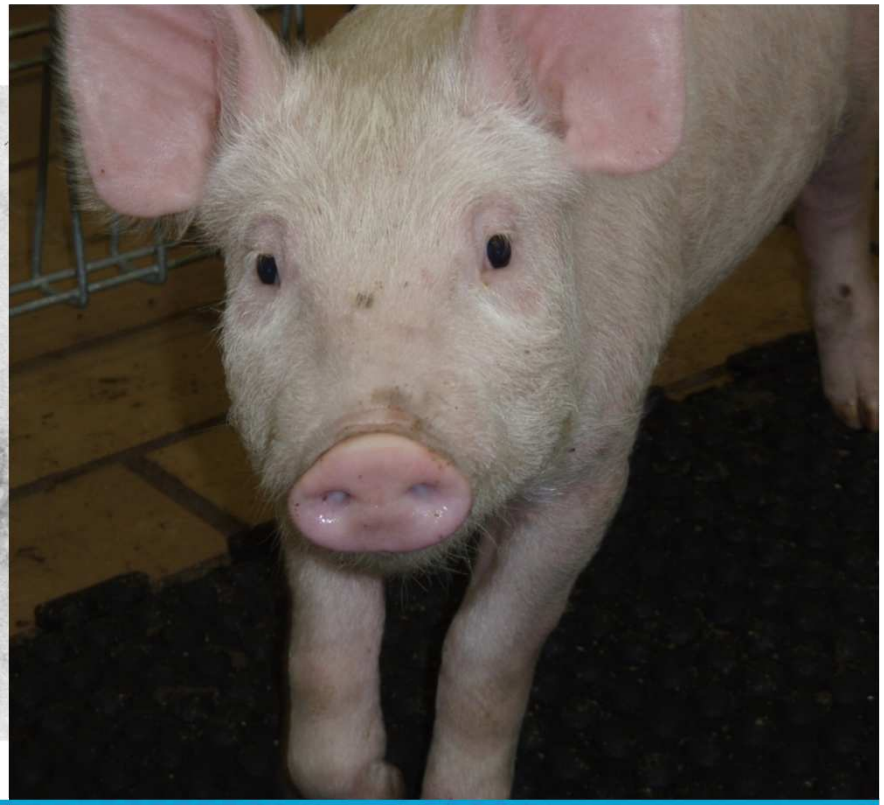

Ebola Reston virus infection of pigs:

disease significance and transmission potential

www.csiro.au

Dr Glenn Marsh

Australian Animal Health Laboratory

CSIRO Livestock Industries

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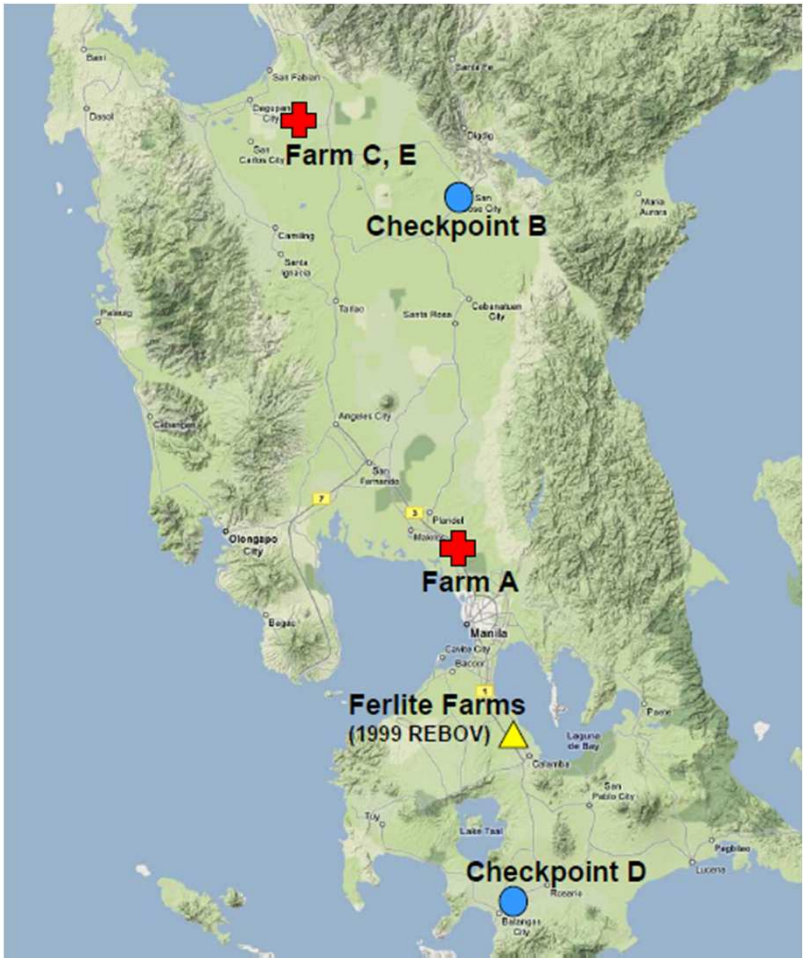
Disease in pigs in the Philippines

http://www.ephilippine.com/wp-content/uploads/2007/12/philippines-map.gif



Barrette, *et al. Science* 2009. **325:** 204 - 206

Summary of disease outbreak in Philippines

- Ebola-Reston is endemic to the Philippines.
- From September 2007 through to May 2008, outbreaks of disease were reported on several pig farms in the Philippines.
- Affected pigs ranged from weanlings to adult animals.
- Disease signs included fever, coughing and skin lesions.

Signs of disease in pigs in Philippines outbreak

Skin hemorrhages (ventral side)

Cyanosis of the ears

NOTE: PRRS cases usually have concurrent infections with Hog Cholera or Classical Swine Fever (CSF), Porcine Circovirus 2, Swine Influenza (SI) and Porcine Epidemic Diarrhea (PED)

Skin hemorrhages on the posterior portion and hind legs

Pale consolidated lungs

Pale hemorrhagic kidney

Images obtained from Samuel Animas, Bureau of Animal Industry, Department of Agriculture, Manila, Philippines

Our involvement

- Our assistance was sought in determining whether or not this virus is truly capable of infecting pigs.
- If so, this is an unprecedented emergence of filovirus infection in a new mammalian host.
- Results will help address important biosecurity implications for both livestock and people in the Philippines as well, as the countries that trade with them.

Questions to be answered

1. Can pigs be experimentally infected with Ebola Reston virus?
2. Do pigs experimentally infected show any clinical signs of illness or, is disease subclinical?
3. Is infection localised or does virus become systemic?
4. Can virus be re-isolated from infected animals?
5. Do infected animals shed virus with the potential for transmission to other pigs or other animals?
6. Do infected animals sero-convert to Ebola Reston virus?

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Design of experiment

- 5 week old pigs were housed in open pens with 2 groups of four pigs for each experiment

- Pigs were exposed to 10^6 $TCID_{50}$ Ebola Reston Philippines' virus by two different routes:
    - Group One was exposed by oro-nasally, mimicking exposure by respiratory or direct contact routes.
    - Group Two received virus by subcutaneous injection to mimic potential spread by biting insects or skin wounds.

Monitoring of animals

- Animals were anesthetised every two days for collection of deep nasal, tonsil and rectal swabs and, blood.
- Body weight and rectal temperatures were also measured whilst anesthetised.
- A urine sample was collected from the floor of pens to test for the presence of virus
- Animals will be euthanized 28 days after exposure to virus to look for development of antibody to the virus that would be suggestive of subclinical infection.

Experiment 1 – Disease progression

- Samples were collected from pigs on days 2, 4, 6, 8, 10 and 13.
- Samples include deep nasal, throat and rectal swabs, blood and urine.
- Blood samples were also collected on days 15, 17, 20 and day 28
- Pigs were euthanized at day 28 and a range of tissues collected.
- All samples were screened by qRT-PCR and positive samples tested by virus isolation

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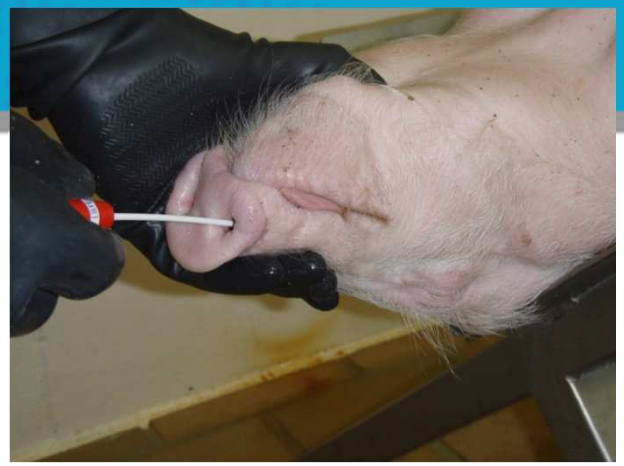
Results – Signs of disease

- No increase in temperature was seen in any pig following exposure to Ebola Reston virus.
- No other signs of illness was observed in any pigs
- Pigs continued to eat and interact, with weight gain consistent with healthy pigs.

Nasal swabs



Real-time RT-PCR results

CDC primer and probe set
(C_T values)

Animal number	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 13
1	U	38.5	29.5 +	27.1 +	31.2 +	U	U
2	U	U	37	26 ++	30.4 +	U	U
3	U	U	26.3 +	22.1 +++	U	U	U
4	U	34.1 +	25.4 ++	23.5 +++	U	U	U
5	U	U	U	U	U	U	U
6	U	U	U	U	U	U	U
7	U	U	U	U	U	U	U
8	U	U	U	U	U	U	U

Animals 1-4 – oro-nasal exposure, 5-8 – subcutaneous
+ indicates virus isolation

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Tonsil swabs

Real-time RT-PCR results

CDC primer and probe set
(C_T values)

Animal number	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 13
1	U	38	U	31 +	U	U	U
2	U	U	35.9	26.9 ++	U	35	U
3	U	U	U	28.3 +	U	U	U
4	U	U	U	U	U	U	U
5	U	U	U	U	U	U	U
6	U	U	U	U	U	U	U
7	U	U	U	U	U	U	U
8	U	U	U	U	U	U	U

Animals 1-4 – oro-nasal exposure, 5-8 – subcutaneous
+ indicates virus isolation

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Summary of Results

- Only pigs exposed to virus by the oro-nasal route shed detectable virus.
- Infection seem to be localised and contained to the upper respiratory tract.
- Infection was of short duration, with shedding occurring from days 2 to 8.
- No signs of illness was seen in any pigs.
- Systemic or blood-borne spread of the virus was not observed in any animal.
- Group Two pigs were not secondarily infected by respiratory droplets or aerosols from the infected Group One pigs in the adjacent pen.

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Serology (NP ELISA)

Pig	Day post infection										
	0	2	4	6	8	10	12	15	17	20	28
1	<100	<100	<100	<100	400	800	800	800	3200	1600	3200
2	<100	<100	<100	<100	<100	200	800	800	400	100	100
3	<100	<100	<100	<100	200	100	100	800	400	100	400
4	<100	<100	<100	<100	<100	200	200	200	400	<100	100
5	<100	<100	<100	<100	200	3200	3200	3200	3200	3200	NT
6	<100	<100	<100	<100	800	12800	12800	12800	12800	6400	NT
7	<100	<100	<100	<100	800	3200	3200	3200	1600	1600	NT
8	<100	<100	<100	100	800	800	1600	800	1600	1600	NT

It is interesting that the intranasal inoculated pigs showed a reduced immune response and shedding (pigs 1-4) where as the IP challenged pigs (pigs 5-8) showed no signs of infection but mounted a robust immune response.

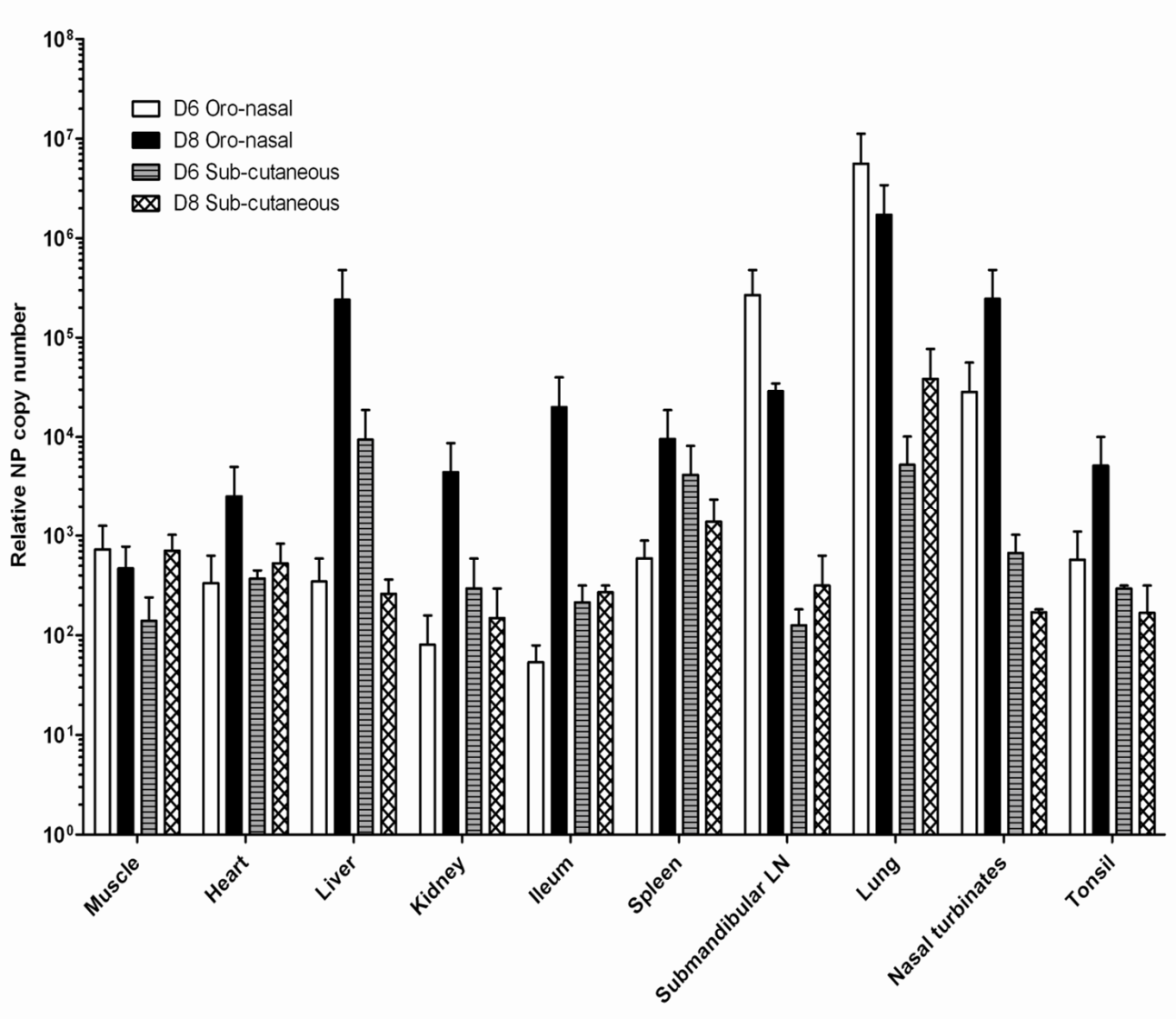
Animals 1-4 – oro-nasal exposure, 5-8 – subcutaneous

Experiment 2 – Virus localisation

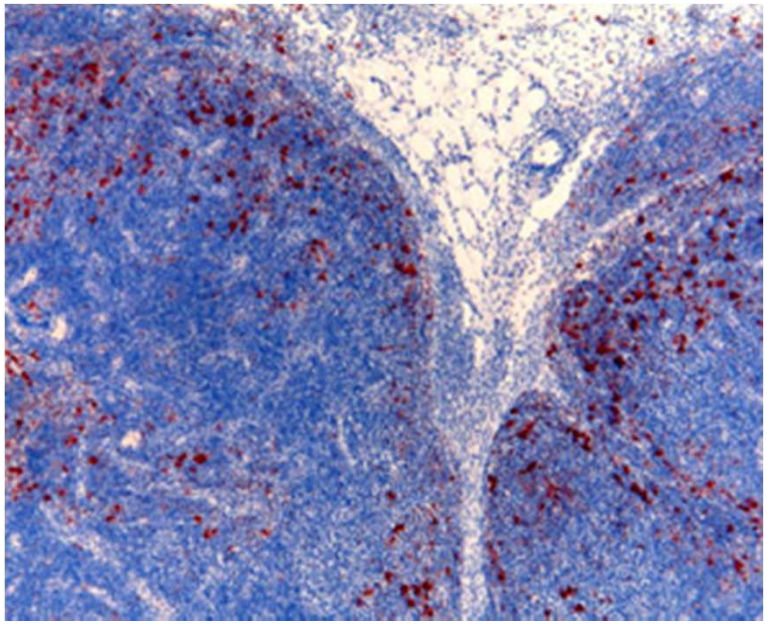
- Based on outcome of Study 1, pigs were similarly exposed to10^6 $TCID_{50}$ of REBOV euthanized on days 6 and 8.

- A range of tissue samples were collected for qRT-PCR, virus isolation and histology.

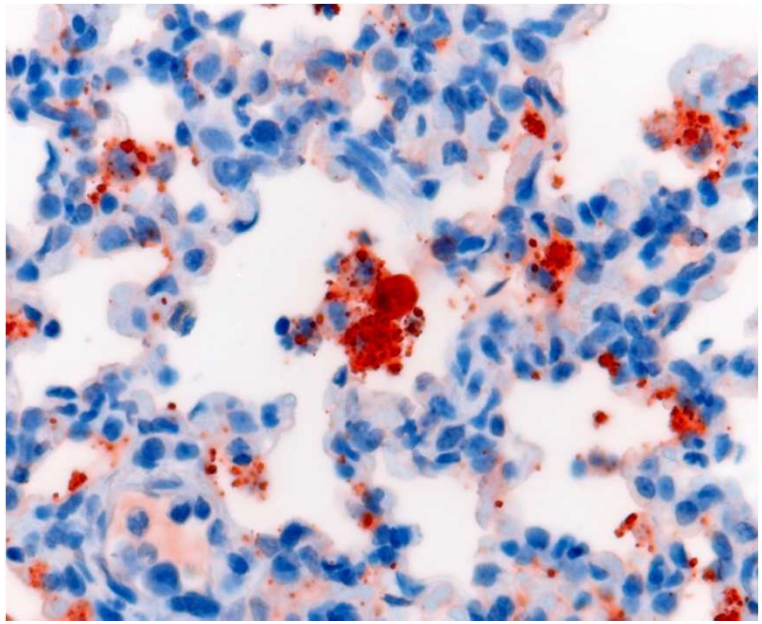
Results – qRT-PCR



Histology



Lymph node of pig following oronasal challenge



Lung of pig with dense viral antigen staining (red) of alveolar macrophages

Conclusions

- Variable results can been seen following experimental challenge of pigs with REBOV
- Presence of pre-existing underlying respiratory infections may allow increase in viral spread
- There is potential for exposure via respiratory secretions, blood and infected tissue
- Farm veterinary and abattoir workers are at greatest risk of exposure

Future directions/Questions

- **Attempt co-infection studies with PRRS and Porcine circovirus**
- **Attempt in-contact transmission experiment??**

- What changes in Ebola Reston are necessary to make a virus that is highly pathogenic in pigs or humans?
- Would these changes occur naturally is the virus is allowed to circulate sub-clinically in pigs in the Philippines?

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CDC – Special Pathogens Branch
USDA Plum Island, USA