Programs to develop and evaluate next generation vaccines for bTB

(models and reagents)

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Relevant Models

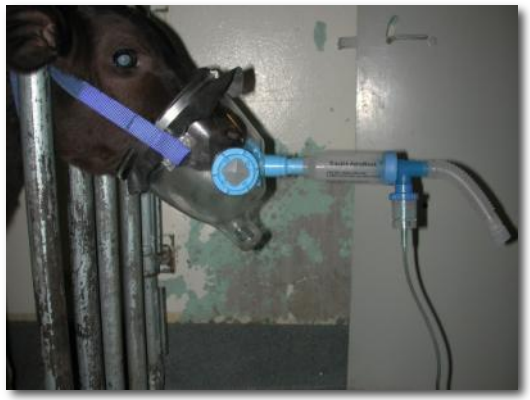
Cattle: DTH, CMI, IFN-γ (Bovigam), poor antibody response

Cervids: DTH (? accuracy), moderate antibody response

Camelids: poor DTH, good antibody response

Eurasian Badgers: Poor DTH, moderate CMI, antibody to MPB83 correlates w/bacterial load & ability to transmit, poor granuloma formation

Ferrets: DTH, good antibody response, moderate granuloma formation,



Preventing bTB Transmission
(natural route infection)

For various reasons (e.g. no sneezing/coughing, poor reagent availability), standard small animal aerosol infection models are not useful for studying transmission



Transmission models under study


Needed Tools and Reagents

Diagnostics: Is the animal infected or vaccinated?

Vaccines: Does the vaccine elicit a response and is this response protective?

Correlations to:

- **Pathology**: post-vaccination infection versus disease
- **Protection**: prevent, cure or prevent transmission?
- **Infection**: Active vs latent? Progressive, resolving, or cured?

Correlates of infection/Protection

During study

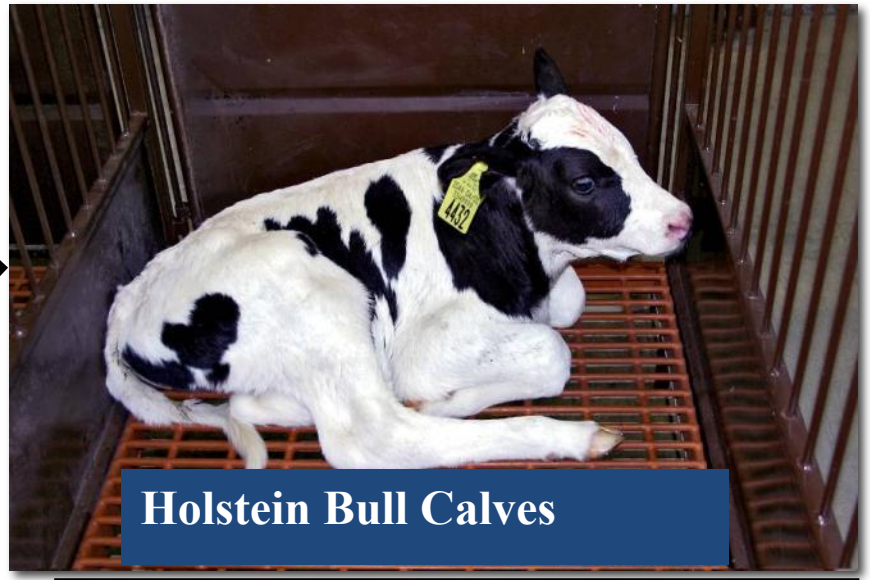
- **DTH (skin test)** -- indicative of prior exposure to *Mycobacterium* spp. but is NOT indicative of disease severity or protection elicited by vaccination
- **Immune responses (blood and BAL)**
  - **IFN-γ responses** -- especially to specific antigens such as ESAT-6/CFP10, but PPDb also is useful. Are indicative of infection but do not necessarily correlate to protection elicited by vaccination
  - **Patterns of response (multi cytokine / chemokine / etc. profile)** – qRTPCR
  - **Humoral response** – to specific vaccine antigens
  - **Central Memory Responses (TcM**) – correlates to reduced bacterial burden and reduced pathology
  - **IL-17** – correlates to pathology, pre-challenge responses may also correlate to protection
- **Bacterial culture** – throat swab, nasal wash, feces, BAL
  - Quantitative (CFU)
  - Qualitative (MGIT)
- **Bacterial PCR** – throat swab, nasal wash

Post-study

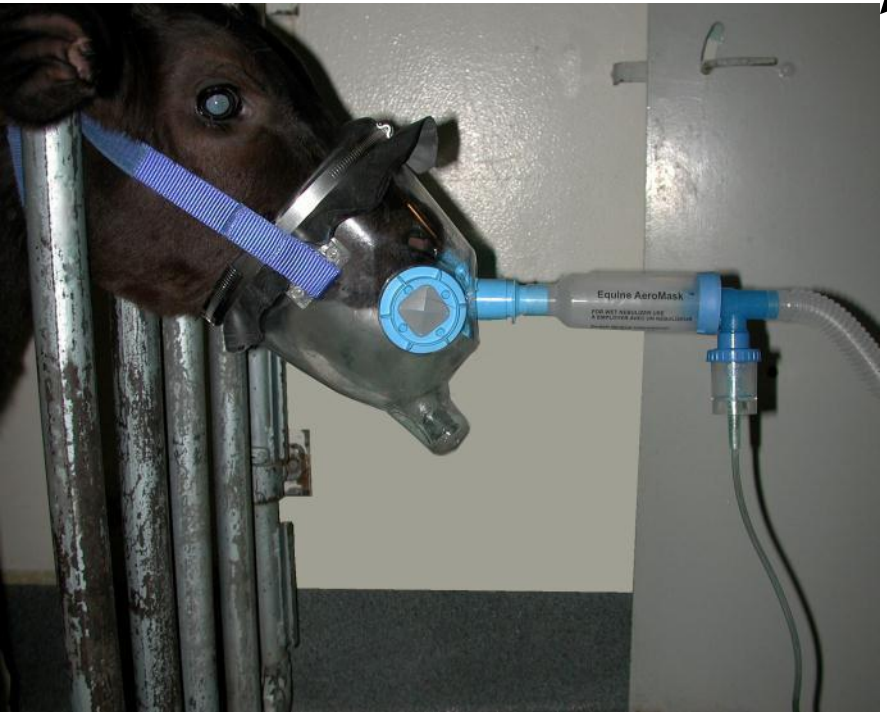
- **Organ pathology**
- **Organ bacterial culture**

Neonatal Calf Vaccine Model

Vaccinate (2 wks of age), n ≃ 10 /group

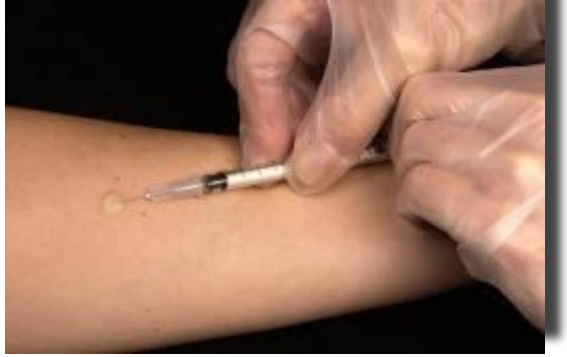


Challenge (10^3 CFU *M. bovis* 95-1315, 3.5 months of age)



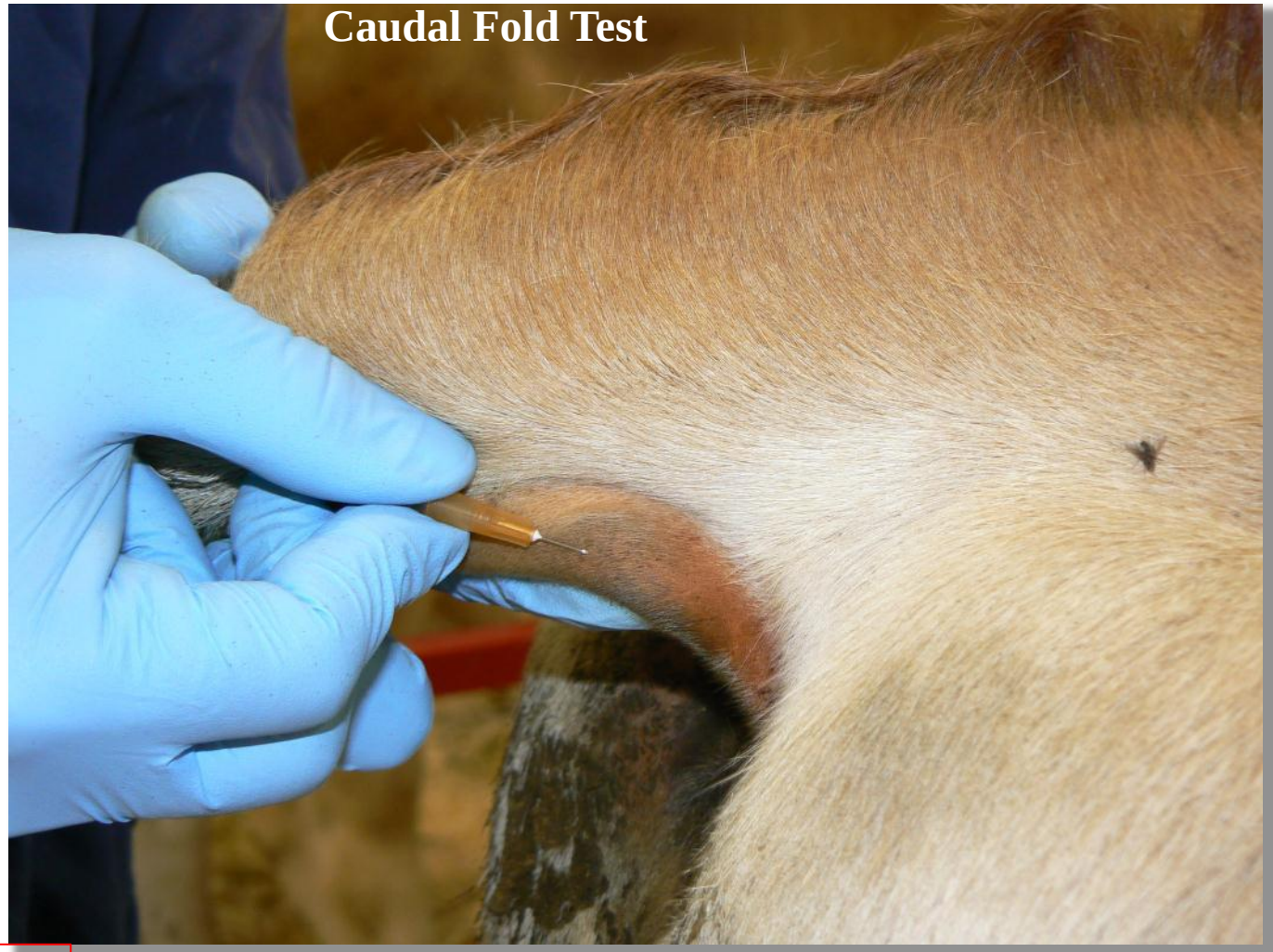
Necropsy (8 months of age)

Data and images by Dr. Ray Waters



bTB DTH

Indicative of exposure to *Mycobacterium* spp. but is NOT indicative of disease severity or protection elicited by vaccination



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IFN-γ Response to PPDb does not always correlate to Pathology; however, it is a good correlate to Infection

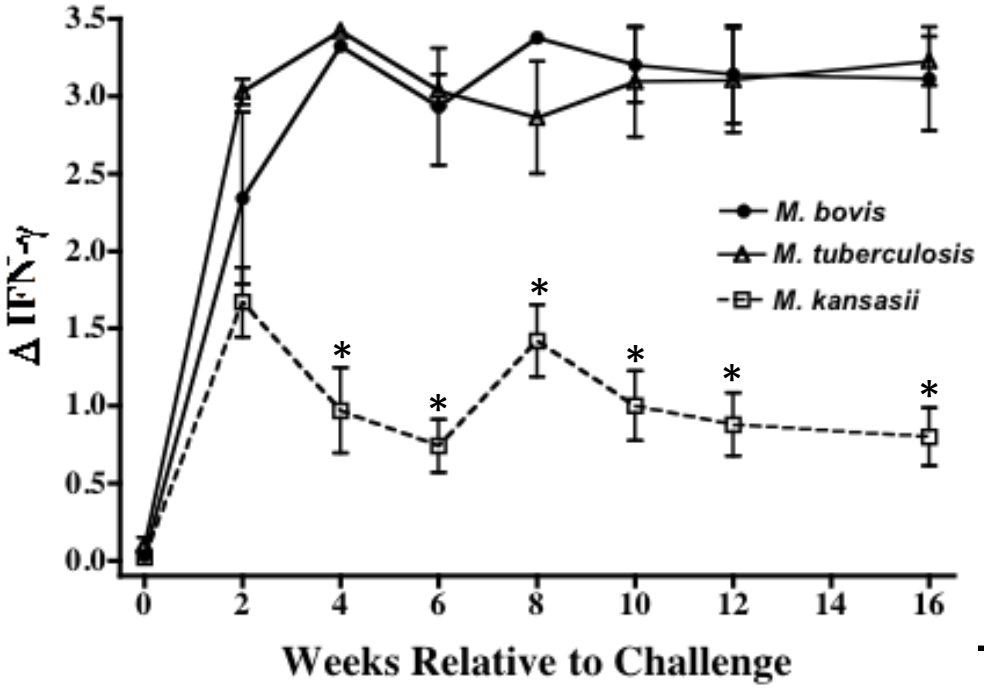
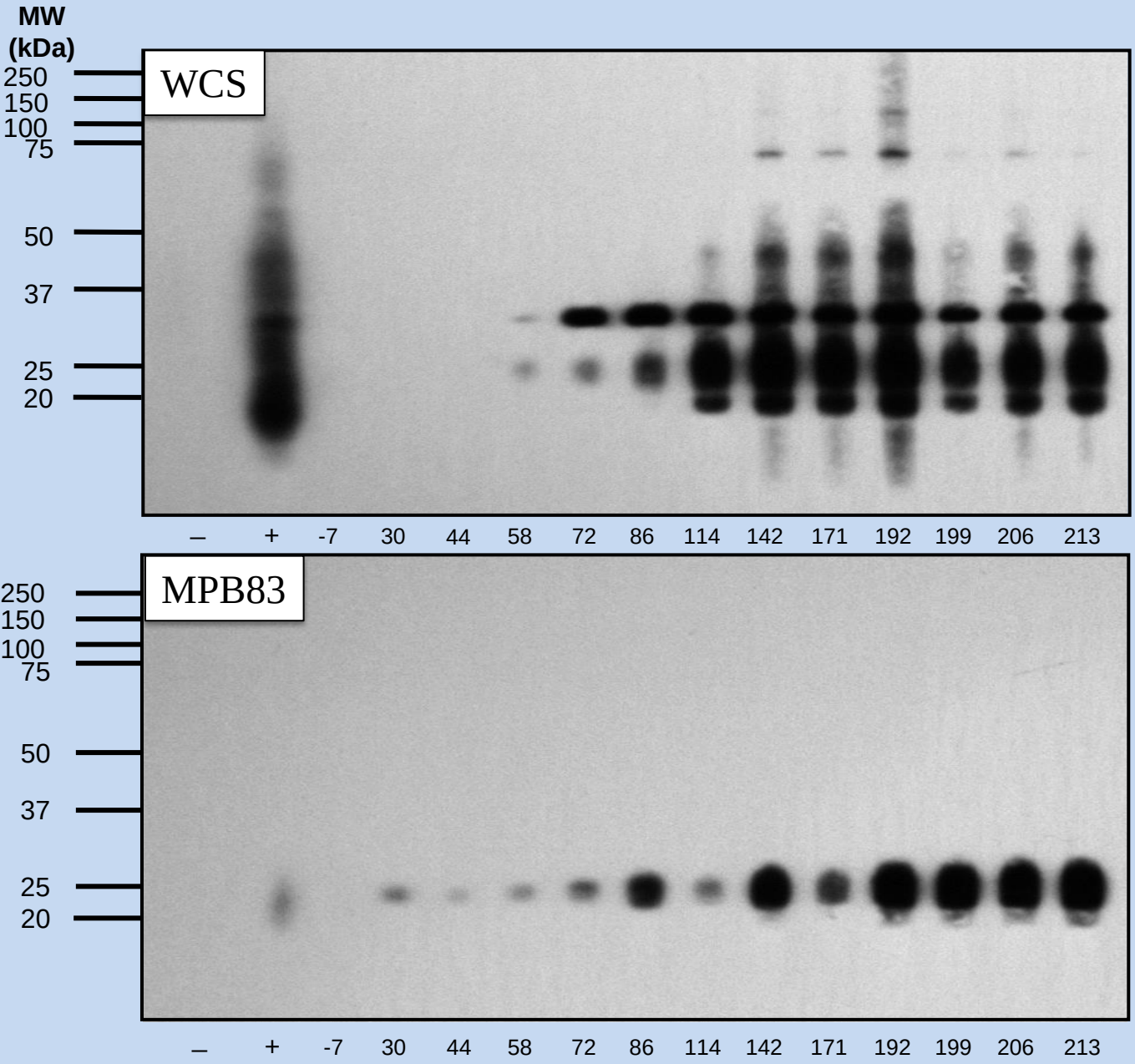


Table 1. Diseaseexpressionupon mycobacterial inoculation.

Group	Gross Pathology[a]	Culture[b] *
M. bovis	All positive	27.2 ± 7.3
(n = 5)		
M. tuberculosis	All negative	13.9 ± 5.5
(n = 5)		
M. kansasii	All negative	0 ± 0
(n = 4)		

Data and images by Dr. Ray Waters



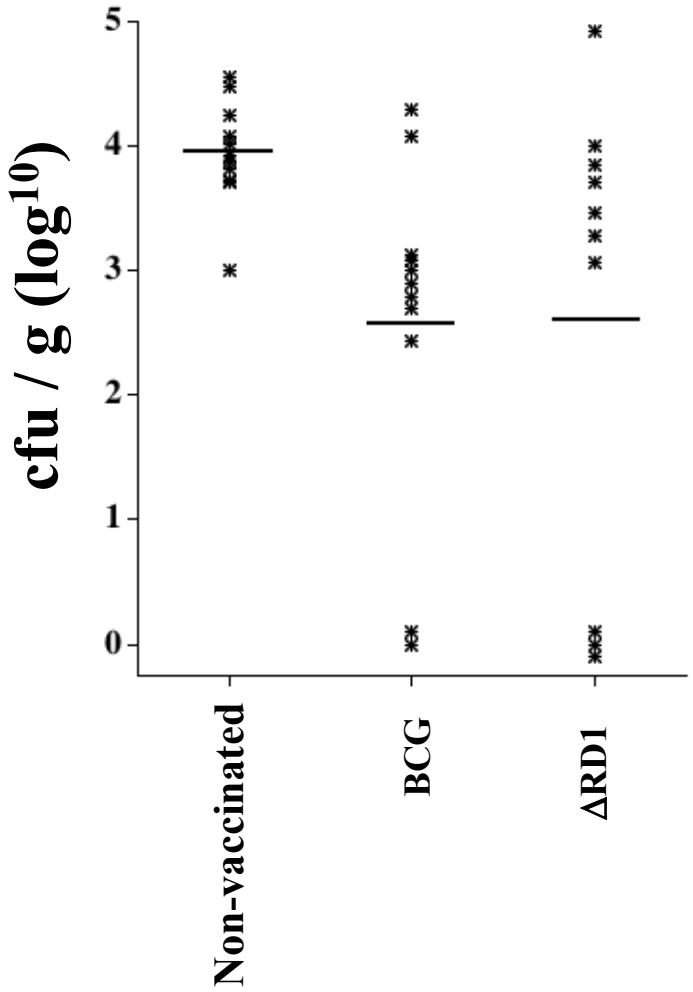
Data and images by Dr. Ray Waters

Early and sustained antibody response does not always correlate to Pathology; however, it is a good correlate to current or prior Infection

- ***M. bovis* strain 1315 whole cell sonicate (WSC)**
- ***M. bovis* strain 1315 MPB83 purified protein**

Quantitative Culture

Tracheobronchial lymph node



Qualitative Culture

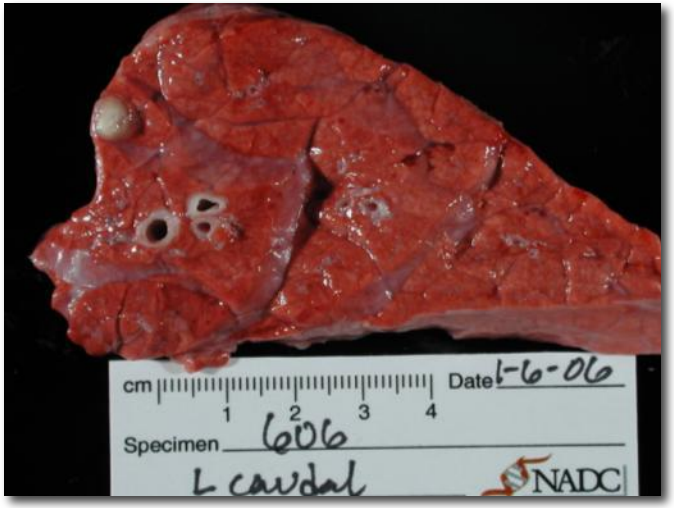
Tracheobronchial lymph node

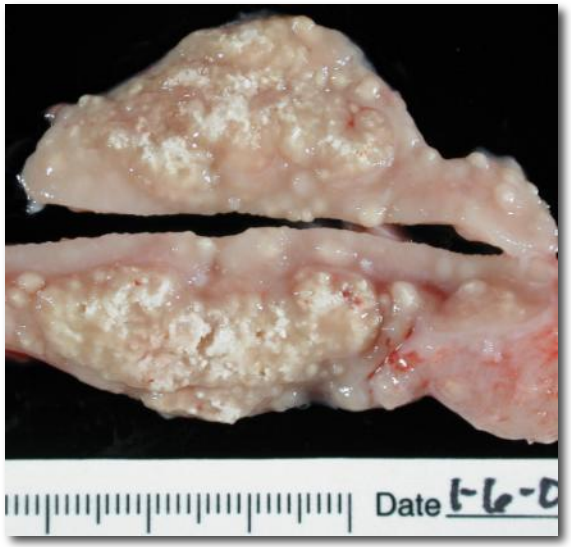
Animal (Non-vaccinated)	MGIT TLN	Animal (BCG-vaccinated)	MGIT TLN	Animal (ΔRD1-vaccinated)	MGIT TLN
1	+	11	+	21	+
2	+	12	-	22	+
3	+	13	+	23	+
4	+	14	-	24	+
5	+	15	+	25	-
6	+	16	+	26	-
7	+	17	+	27	+
8	+	18	+	28	+
9	+	19	+	29	-
10	+	20	+	30	+

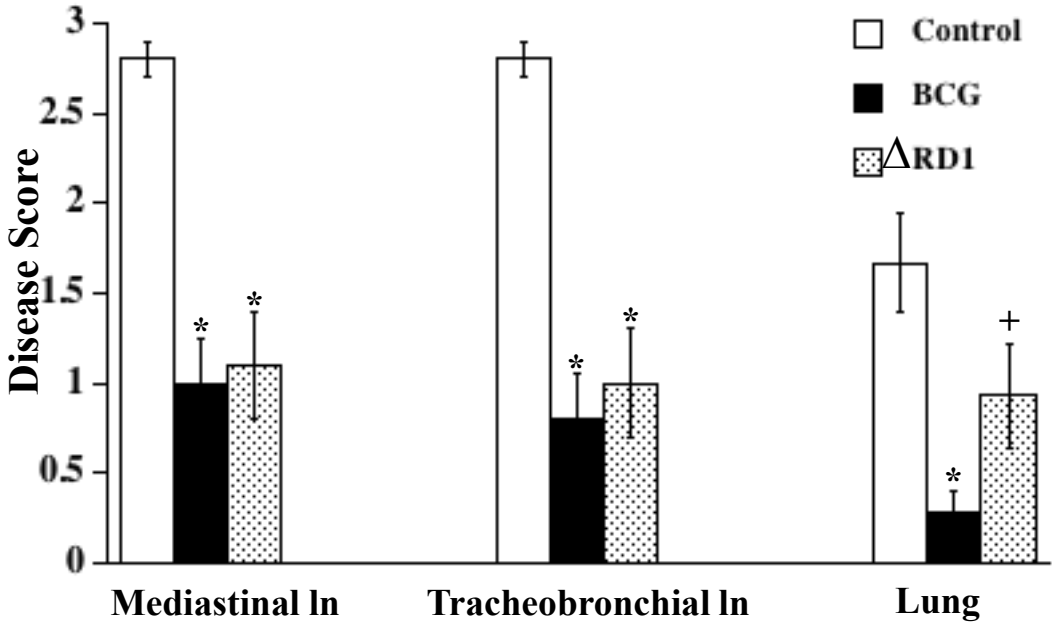
Quantitative and Qualitative assessments provide useful information. Quantitative is more precise when available

Data and images by Dr. Ray Waters

Gross and Histopathology, Disease Scoring







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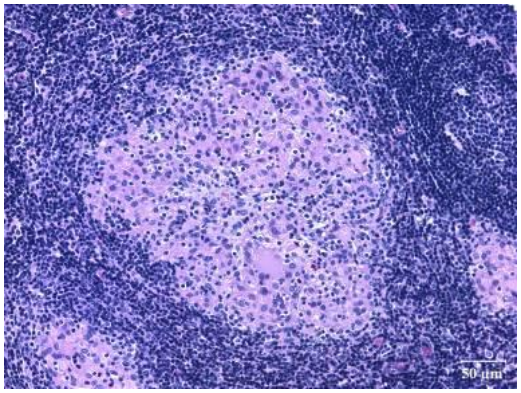
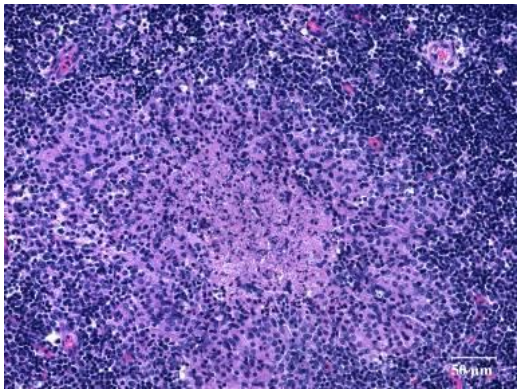
n = 10

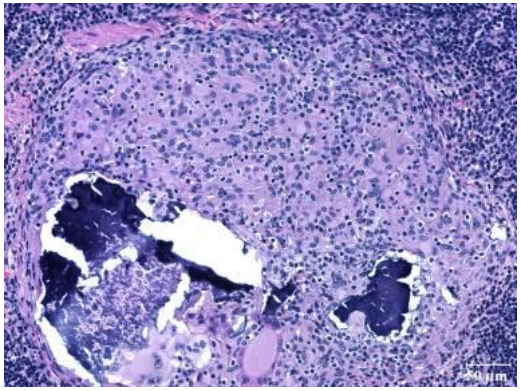
Stage 1. Necrosis absent



Stage 2. Minimal necrosis



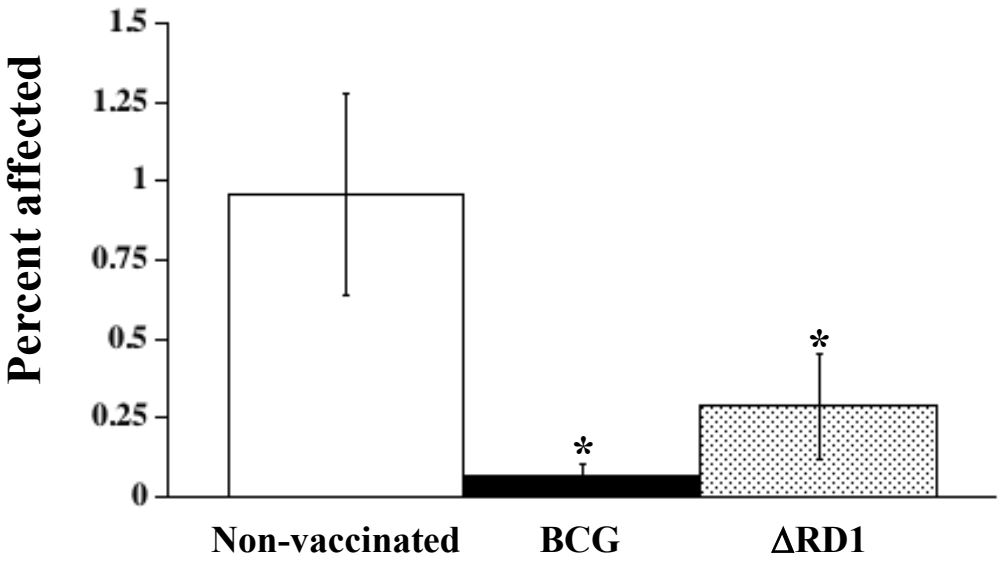
Stage 3. Necrosis



Stage 4. Mineralized

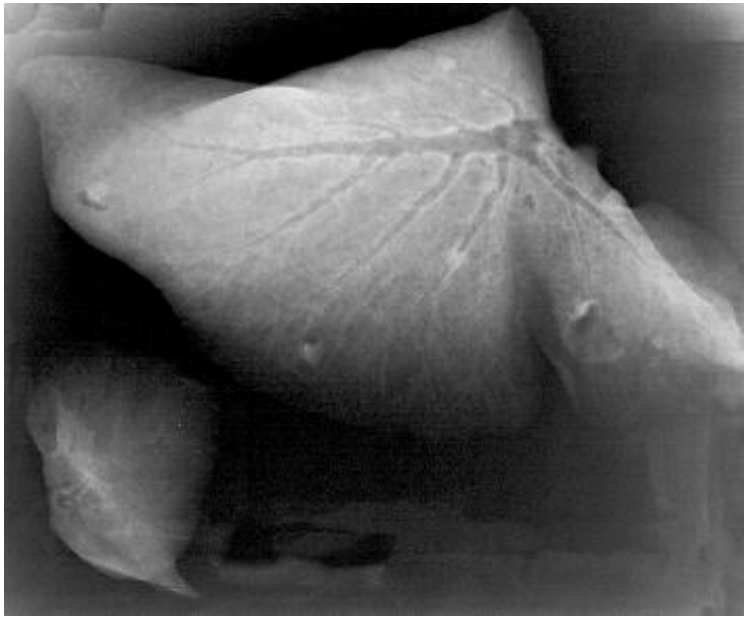
Radiograph Morphometry

Mean ± SEM

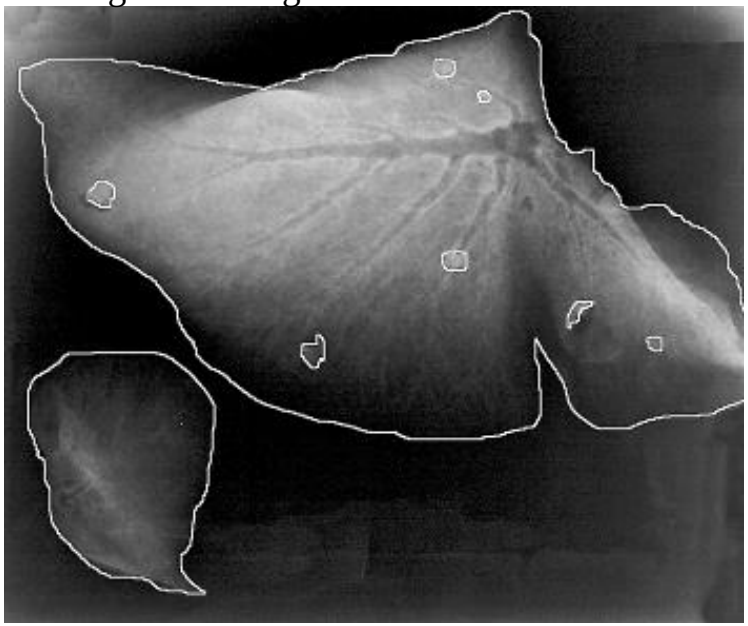


n = 10

Original image



Lungs with margins and lesions outlined



Data and images by Dr. Ray Waters

Opportunities / Relevance, Calf Model

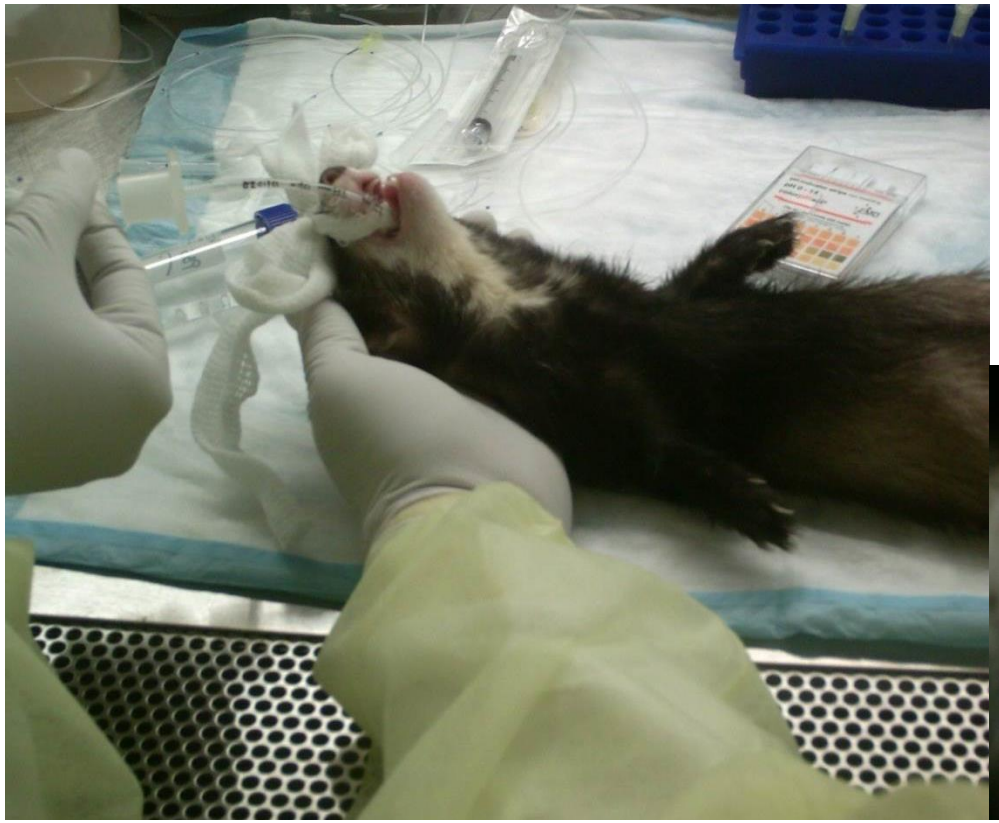
- Large numbers of calves available throughout the year, including neonates
- Pulmonary distribution of lesions with oral/aerosol challenge
- Measurable transmission studies
- Nutritional status can be manipulated
- Additional vaccine safety screen – especially for the neonate
- Long term immunity studies
- Correlates of protection studies
- Field trials possible w/ relevant disease interactions and constraints on platform

Limitations, Calf Model

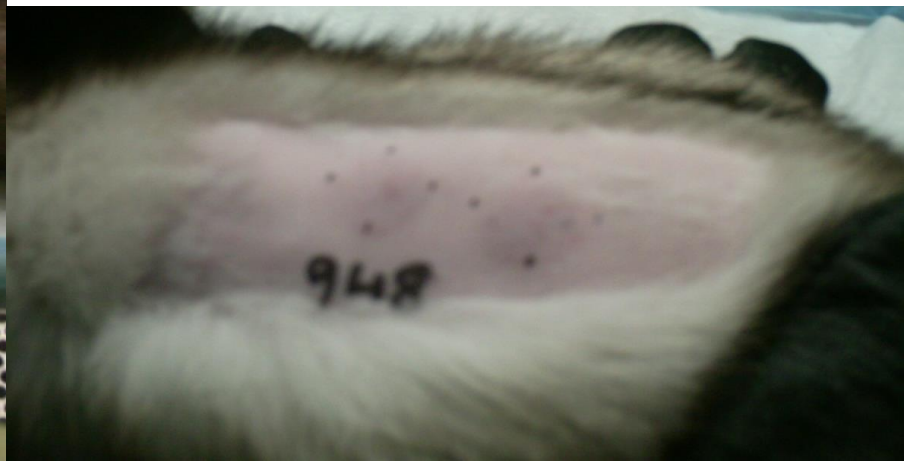
- Costly BSL-3 facilities due to size of animals, 1 to 2 studies per year in AHRC
- Reagent availability now less of a problem

Opportunities / Relevance, Ferret Model

Intratracheal infection with *M.tb* strain Erdman



Skin test results - erythema and induration



Low dose

Wk 2
Wk 4
Wk 7

Mtb

F#	NW MIGT	TS MIGT	URT MIGT	Feces MIGT	St MIGT	Lg MIGT	CFU Lg	Sp MIGT	CFU Sp	Lv MIGT	CFU Lv	CFU LN	Skin test	IgG
13	n/a	n/a	-	-	-	+	+	-	+	n/a	n/a	n/a	n/a	-
14	+	n/a	-	-	-	+	+	+	0	+	n/a	n/a	n/a	+
15	n/a	n/a	-	-	-	+	+	+	+	n/a	n/a	+	n/a	-
16	n/a	n/a	-	-	-	n/a	+	+	+	n/a	+	0	16	+
17	n/a	n/a	+	+?	-	+	+	+	+	+	+	n/a	20	-
18	n/a	n/a	-	-	-	n/a	+	+	+	n/a	0	+	16	-

Medium dose

Wk 2
Wk 4
Wk 7

Mtb

F#	NW MIGT	TS MIGT	URT MIGT	Feces MIGT	St MIGT	Lg MIGT	CFU Lg	Sp MIGT	CFU Sp	Lv MIGT	CFU Lv	CFU LN	Skin test	IgG
7	-	n/a	-	-	-	+	+	+	+	-	n/a	+	n/a	+
8	-	n/a	+?	-	-	+	+	+	+	-	n/a	+	n/a	+
9	-	n/a	+?	+?	-	+	+	+?	+	-	n/a	+	n/a	+
10	n/a	n/a	+?	-	-	+	+	+	+	-	+	+	16	-
11	-	n/a	n/a	-	+	+	+	+	+	-	+	+	16	+
12	-	n/a	n/a	-	-	+	+	+	0	-	0	+	16	+

High dose *Mtb*

Wk 2
Wk 4
Wk 7

F#	NW MIGT	TS MIGT	URT MIGT	Feces MIGT	St MIGT	Lg MIGT	CFU Lg	Sp MIGT	CFU Sp	Lv MIGT	CFU Lv	CFU LN	Skin test	IgG
1	-	+	-	-	-	+	+	-	+	-	n/a	+	n/a	+
2	-	+	+	-	-	+	+	+	+	-	n/a	+	n/a	-
3	+	+	+	-	-	+	+	-	+	-	n/a	+	n/a	+
4	-	+	+	+	+	+	+	+	+	+	+	+	16	-
5	+	+ +	+	++	+	+	+	+	+	-	+	+	13	-
6	-	+	-	-	-	+	+	-	+	-	+	+	13	-

Conclusions

- Ferrets develop acute infection within 4 weeks using low dose installation
- Bacilli are detectable in the URT and nasal secretions of some low dose infected animals by 4 weeks post infection (pi) and in most medium and high dosed animals by 7 weeks pi.
- The PPD skin test is useful for following disease progression

Ongoing

- Long duration transmission study

Opportunities / Relevance, Ferret Model

- Large numbers of ferrets available throughout the year
- Pulmonary distribution of lesions with oral/aerosol challenge
- Potentially measurable transmission studies (ongoing for *Mtb*)
- Nutritional status can be manipulated
- Potential vaccine safety screen (planned for *Mtb*)
- Long term immunity studies (ongoing for influenza)
- Correlates of protection studies
- Reasonable cost; ease of manipulation

Limitations, Ferret Model

- No field studies; need to include a subsequent cow study
- Reagent availability now less of a problem

Mucosal Vaccine Candidate Platforms

Mycobacterium shottsii (Pathvac)

- Naturally cold-adapted/safe
  - grows optimally at 22-26°C; no growth >29°C
  - safe for immunocompromised humans
  - safety tested in mice and guinea pigs
- Natural adjuvant/highly immunogenic
  - cell wall chemistry very similar to Freund's incomplete adjuvant
  - induces humoral and CMI responses
  - can be cultured from nasal tissue (only) for several weeks
  - no pre-existing vector immunity
- Efficacious/easily manipulated genome
  - candidates for *M. tuberculosis* have been protective
- Live mucosal (aerosol) vaccine
- Inexpensive to produce
- Genome stability
  - non-invasive (needle-free)

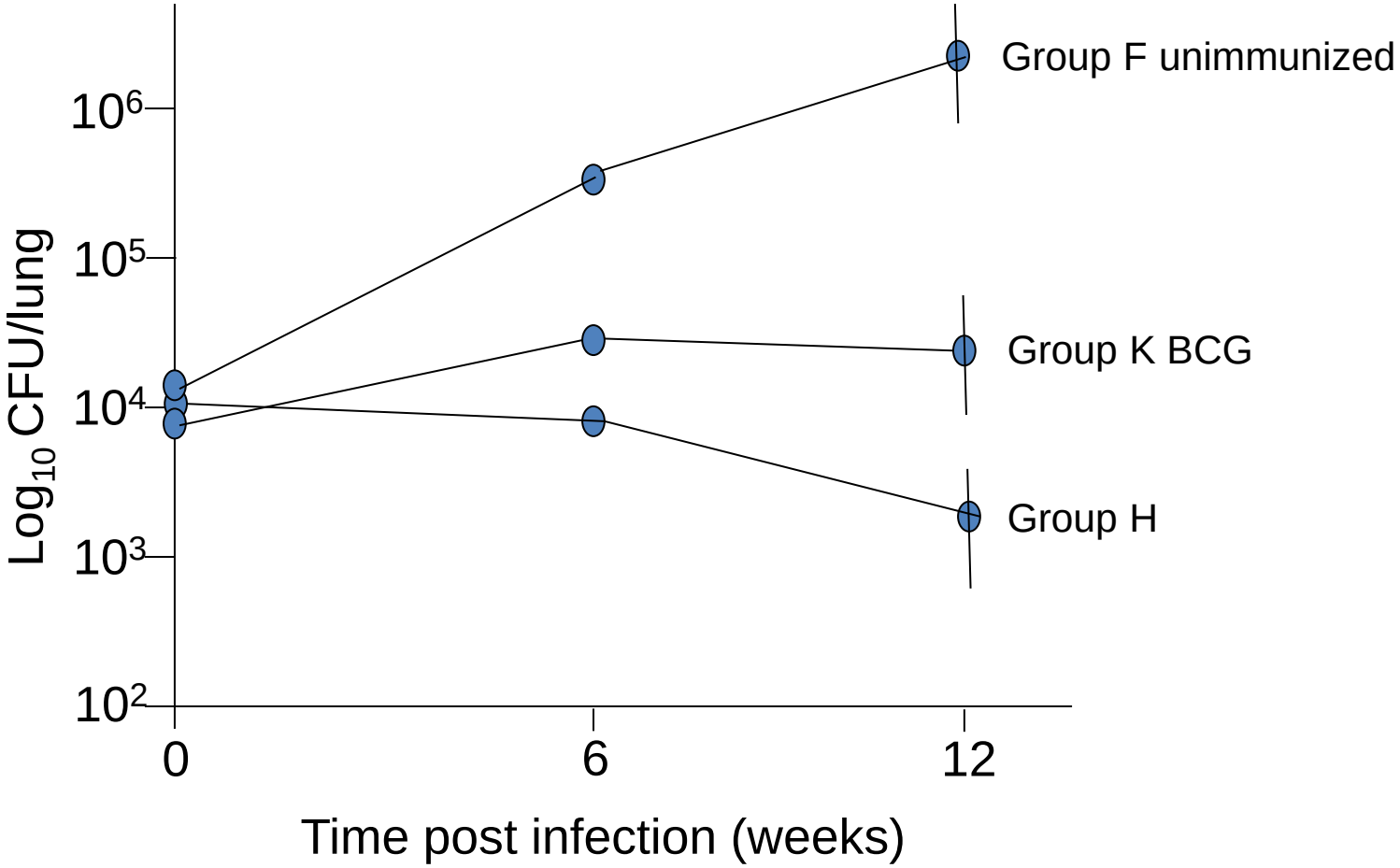
Parainfluenza Virus 5 (PIV5)

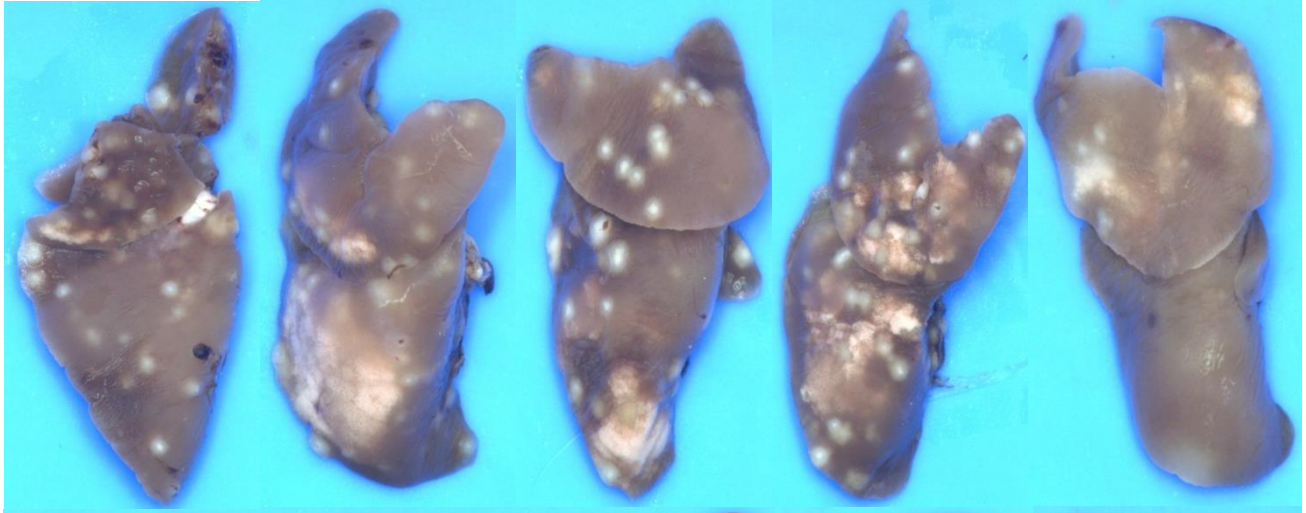
- Safe
  - Safety tested in mice, hamsters, guinea pigs, cotton rats, ferrets, cats, dogs, pigs, horses, monkeys, chickens and humans
- Highly immunogenic
  - induces both humoral and CMI responses
  - can be cultured from nasal tissues and lungs for several weeks
  - no pre-existing vector immunity
- Efficacious/easily manipulated genome
  - candidates for influenza, rabies, respiratory syncytial virus, HIV, Ebola, *Burkholderia mallei*, *Mycobacterium tuberculosis* have been protective
- Live mucosal (aerosol) vaccine
- Inexpensive to produce
- Genome stability
  - non-invasive (needle-free)

Bovine passive immunization

- Can mucosal vaccination prevent transmission to calves?
- Lower lung counts to prevent aerosol transmission
- Use Magpix diagnostic?

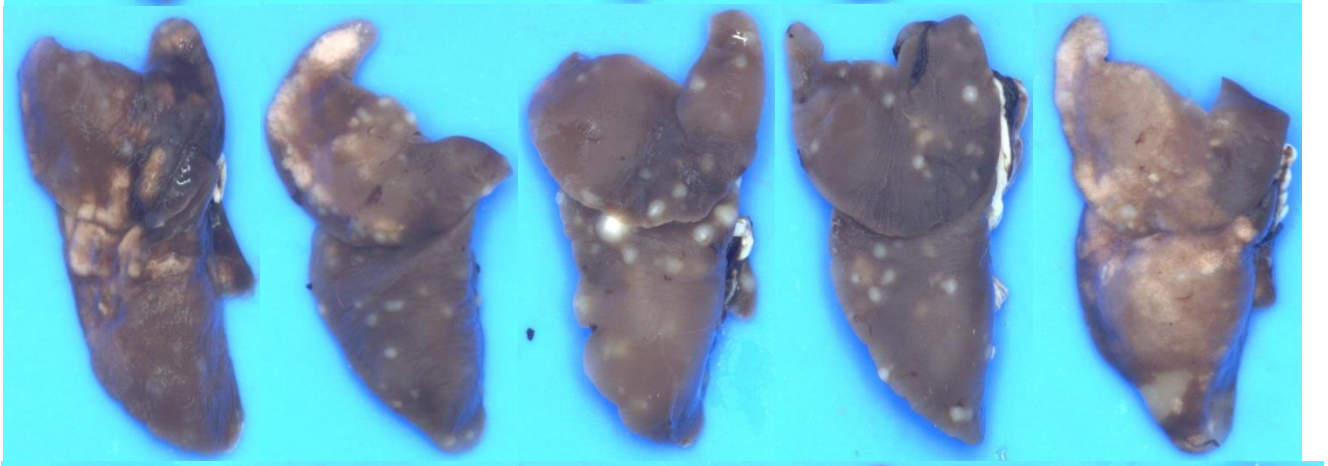
Lung counts


Group F unimmunized
Group K BCG
Group H
10^6
10^5
10^4
10^3
10^2
Log_{10} CFU/lung
0
6
12
Time post infection (weeks)

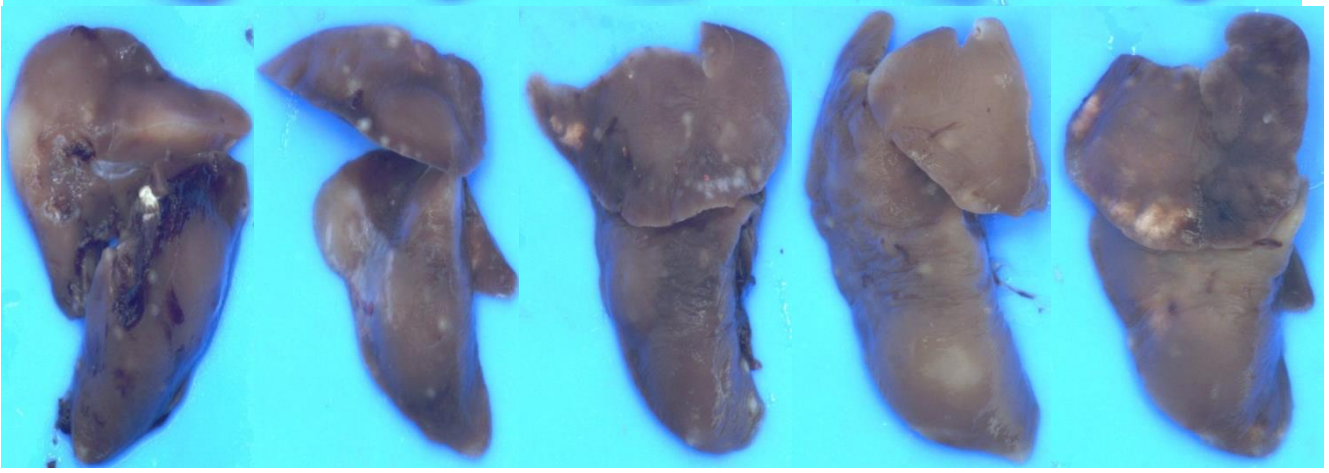


Group F – Unimmunized

6 weeks after infection



Group K - BCG 1 dose i.d.

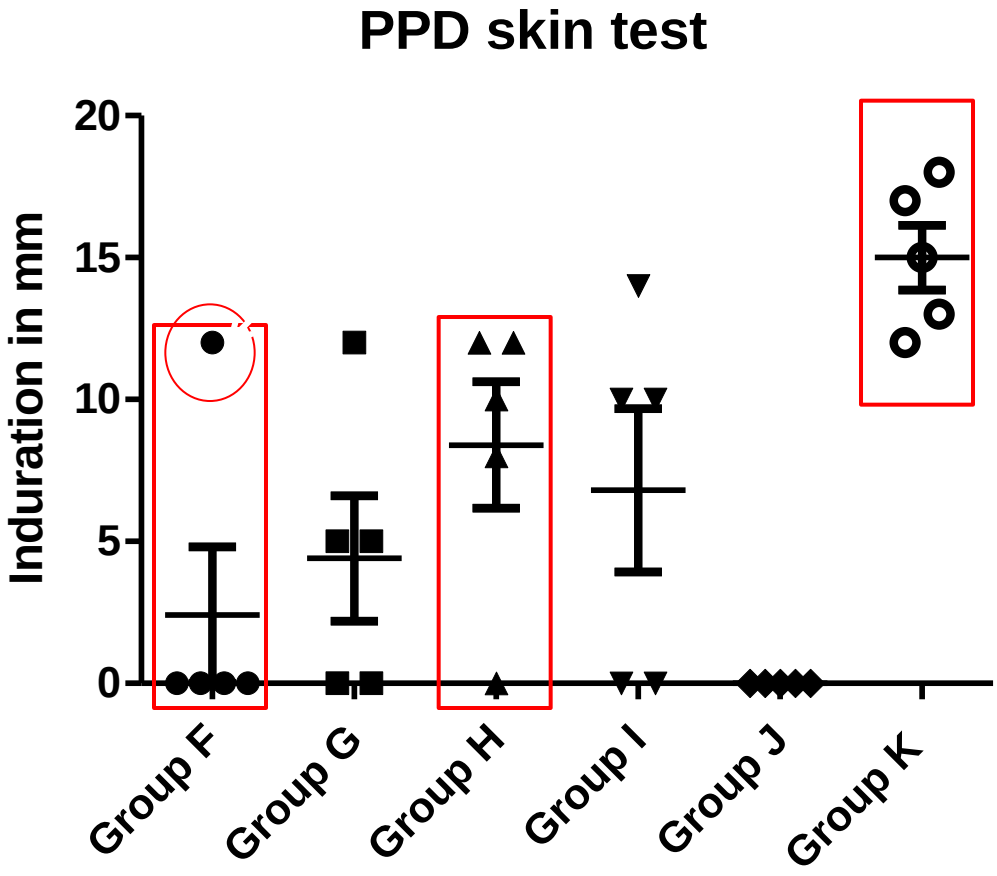


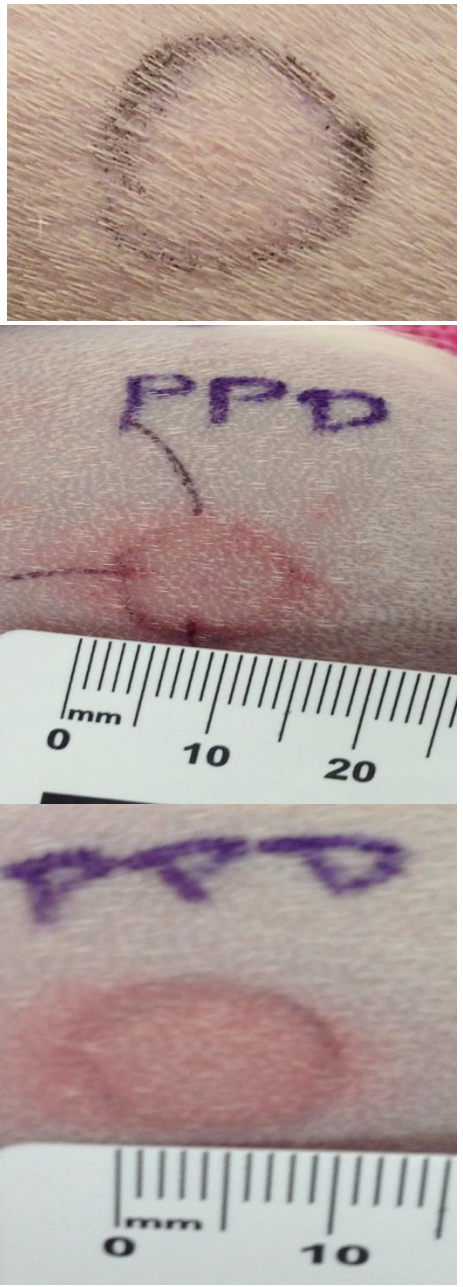
Group H - 2 doses 3 wk apart i.n.

Group F – Unimmunized

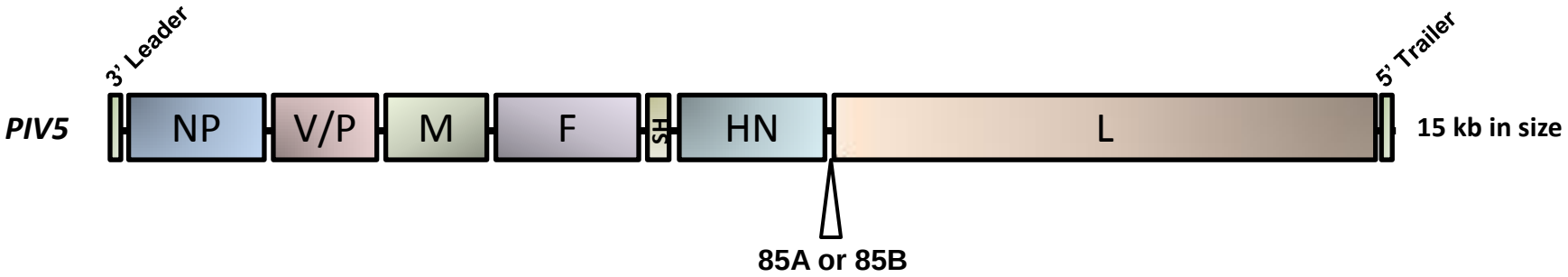
Group H - 2 doses 3 wk apart i.n.

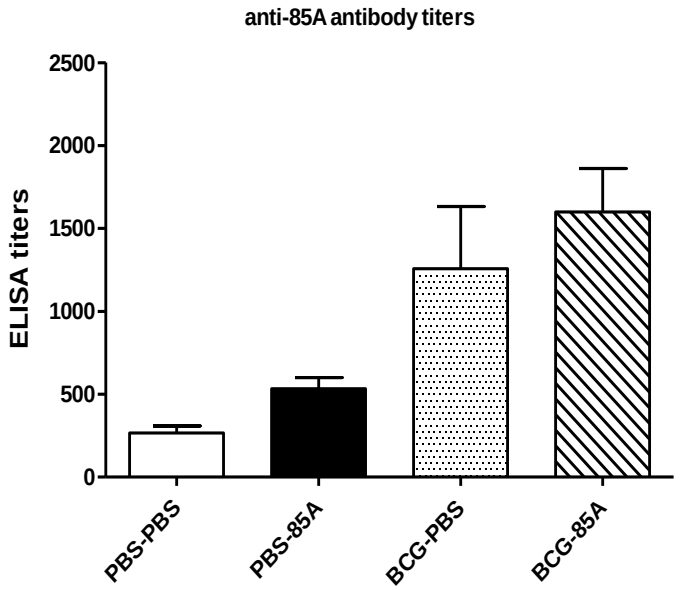
Group K - BCG 1 dose i.d.

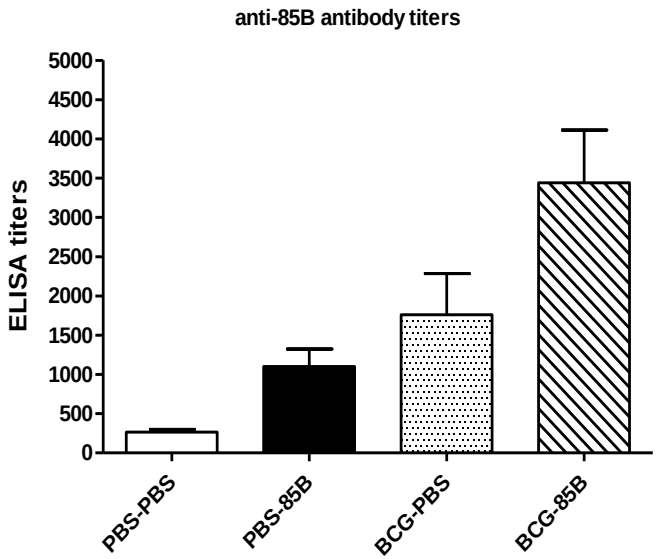




Recombinant PIV5 expressing 85A or 85B of *Mycobacteria Tuberculosis*

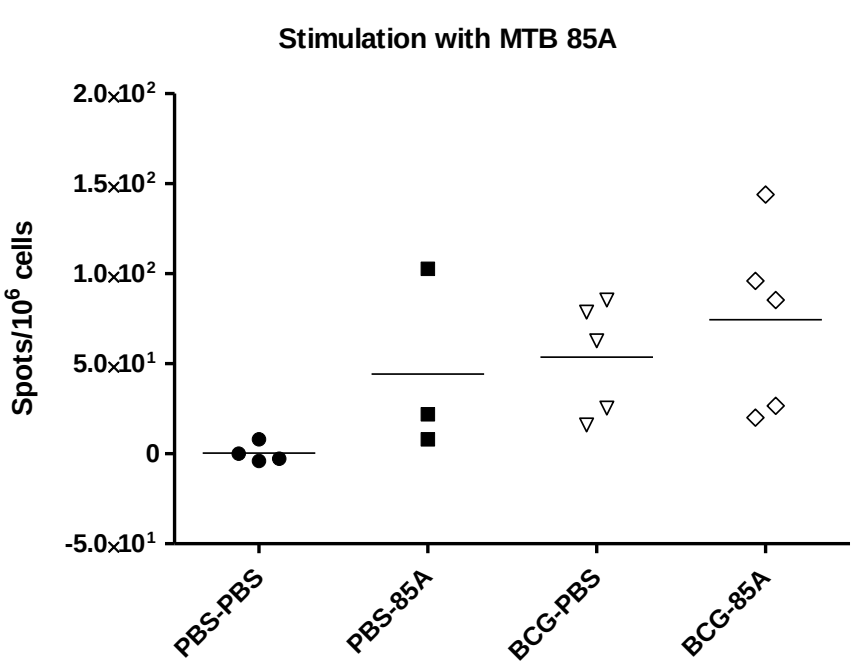


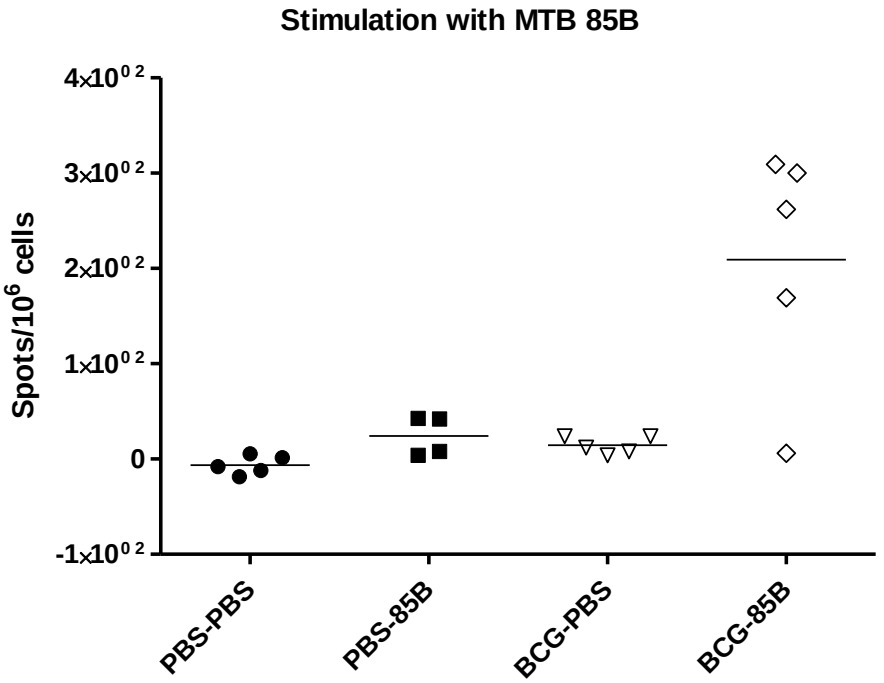




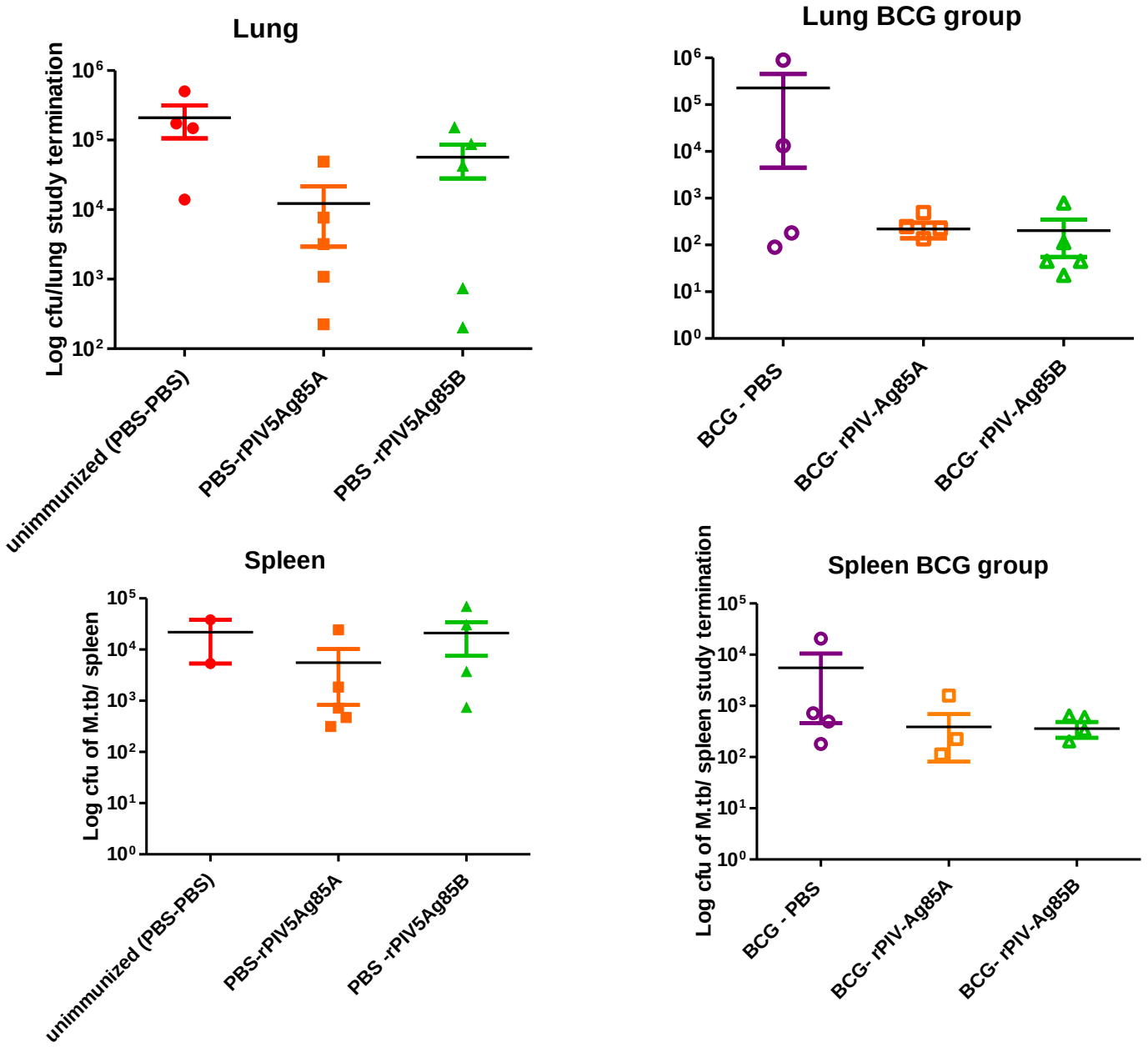
Antibody responses in PIV5-85, 85B and BCG immunized mice.

Cellular immune responses in PIV5-85, 85B and BCG immunized mice.





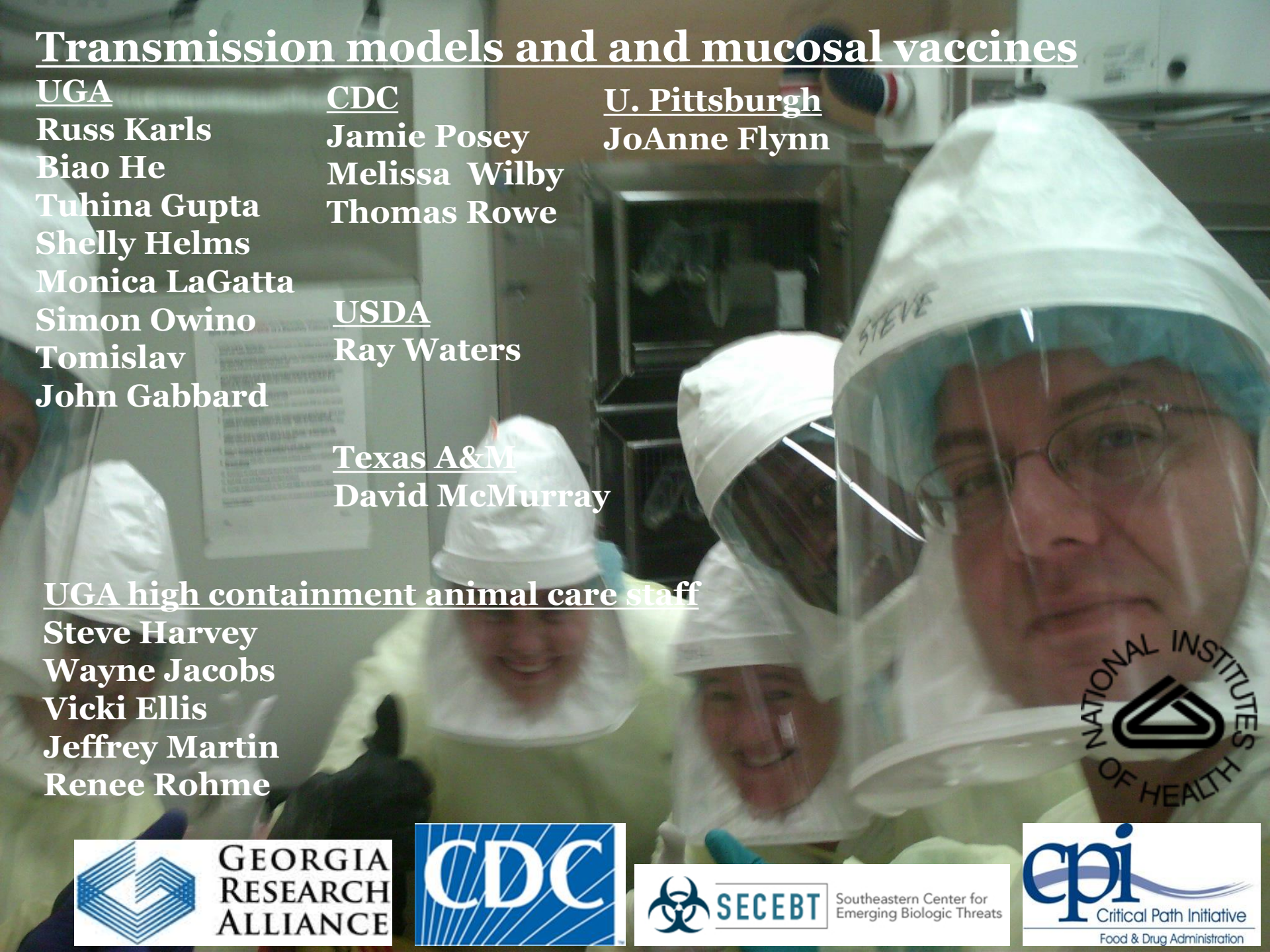
Mycobacterium tuberculosis load in PIV5-85, 85B and BCG immunized mice.



Summary and Future Directions

- Control of bTB may reside in implementation of an effective vaccine program
- Sterilizing immunity by a vaccine may not be attainable
  - our goal for now might be prevention of disease transmission
- Best model for testing vaccine efficacy and disease transmission is the cow,
  - an appropriate small animal may be the ferret
- Two new mucosal vaccines that decrease transmission potential are available and under investigtion

Transmission models and and mucosal vaccines



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Renee Rohme

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cpi Critical Path Initiative
Food & Drug Administration