TARGET Report – Study performed by Drs. Lan Ly and David McMurray at Texas A&M University; histopathology and report prepared by Dr. Paul Converse at Johns Hopkins University.

Experiment in guinea pigs using mutants and parental strain obtained from Drs. Suman Laal, Krishna Singh, and colleagues.

Strains

1. H37Rv
2. H37Rv Δ*PE-PGRS51* (Rv3367)
3. H37Rv Δ*PTRP* (Rv0538)

Goals:

- Assess bacterial burden on day 1 after infection in entire lung (5 lobes)
- Assess bacterial burden on weeks 3 and 6 after infection in lung and spleen
- PPD skin test at 3 and 6 weeks after infection
- Lung histopathology at 3 and 6 weeks after infection
- Serum collected at weeks 3 and 6 after infection and sent to Dr. Laal

Day 1 results after aerosol exposure to 2 x 10^7 bacilli/ml

The entire lung (5 lobes) was homogenized in 9 ml of sterile saline and 2 ml. were plated onto 20 plates to determine implantation. Δ*PTRP* may have an implantation defect.

Strain	**Mean Total CFU in lung (N=5)**	**Log_{10} CFU ± SEM**
H37Rv	211	2.32 ± 0.04
H37Rv Δ*PE-PGRS51* (Rv3367)	95 (one sample contaminated)	1.94 ± 0.10
H37Rv Δ*PTRP* (Rv0538)	6.6 (two animals below detection limit, 4)	0.78 ± 0.09

Week 3 results after aerosol exposure to 2 x 10^7 bacilli/ml

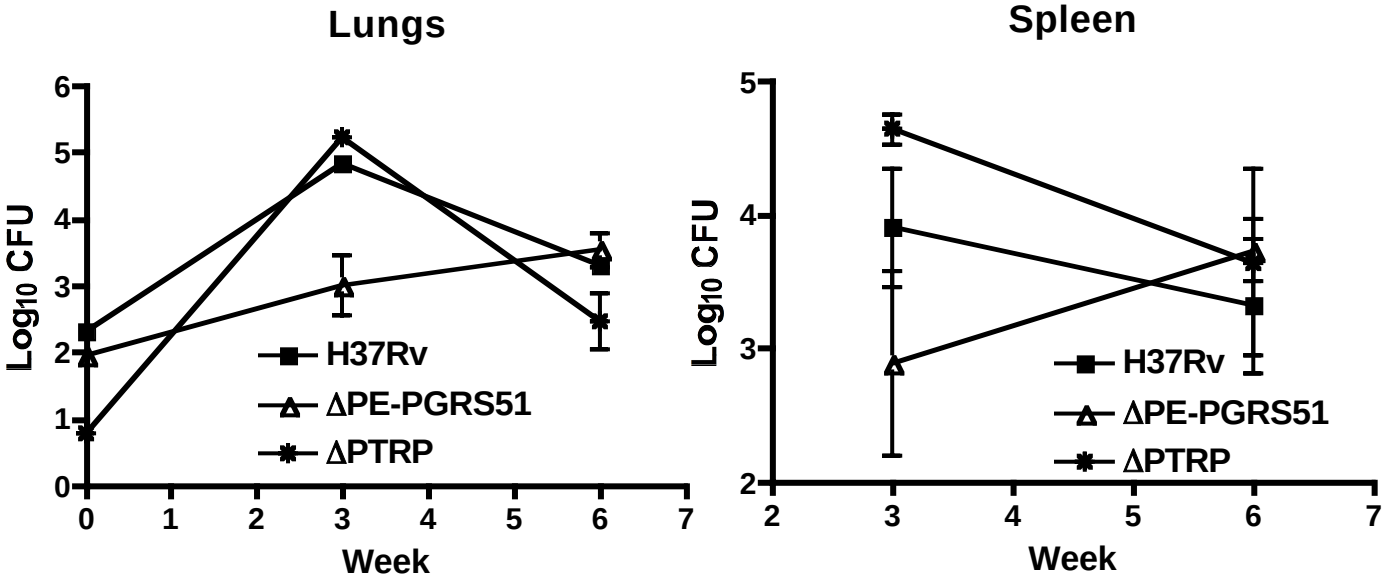
Sera were collected and PPD skin tests performed; Lungs and spleens were homogenized for bacteriology and remaining tissues were taken for histopathology.

Strain	**Log_{10} cfu in lung (N=5) ± SEM**	**Log_{10} cfu in spleen (N=5) ± SEM**
H37Rv	4.83 ± 0.07	3.90 ± 0.44
H37Rv Δ*PE-PGRS51* (Rv3367)	3.00 ± 0.46 (one animal below detection limit, 1.35)	2.89 ± 0.7 (2 animals below detection limit, 1.35)
H37Rv Δ*PTRP* (Rv0538)	5.22 ± 0.06	4.64 ± 0.12

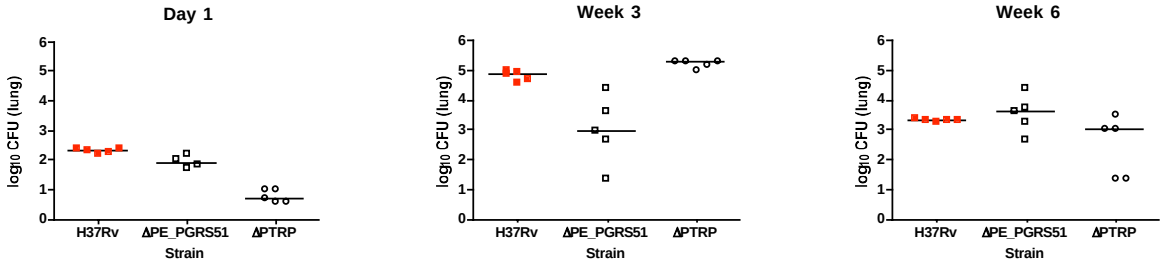
Week 6 results after aerosol exposure to 2 x 10^7 bacilli/ml

Sera were collected and PPD skin tests performed; Lungs and spleens were homogenized for bacteriology and remaining tissues were taken for histopathology.

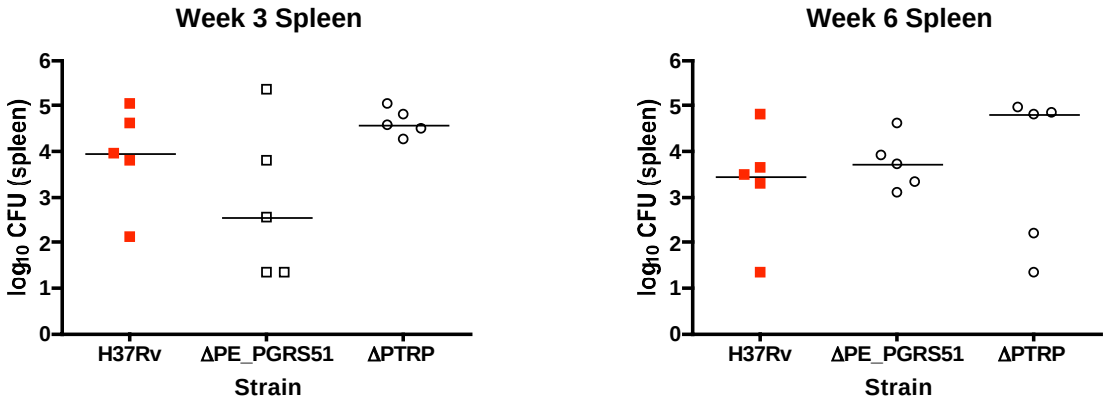
Strain	**Log_{10} cfu in lung (N=5) ± SEM**	**Log_{10} cfu in spleen (N=5) ± SEM**
H37Rv	3.31 ± 0.02	3.32 ± 0.50 (one animal below detection limit)
H37Rv Δ*PE-PGRS51* (Rv3367)	3.54 ± 0.26	3.73 ± 0.23
H37Rv Δ*PTRP* (Rv0538)	2.46 ± 0.41 (2 animals below detection limit, 1.35)	3.64 ± 0.69 (one animal below detection limit, 1.35)



Lung CFU displayed as scatter plots

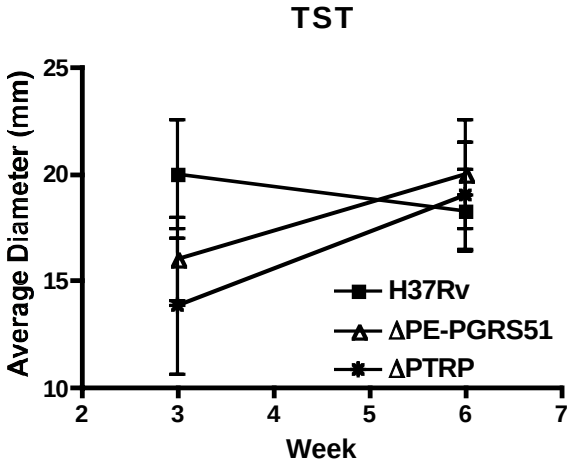


Spleen CFU displayed as scatter plots



PPD Skin tests at 3 and 6 weeks

Strain	Week 3	Week 6
H37Rv	20 ± 2.6	18.3 ± 1.9
H37Rv Δ*PE-PGRS51* (Rv3367)	16 ± 2.0	20 ± 2.6
H37Rv Δ*PTRP* (Rv0538)	13.8 ± 3.2	19 ± 2.5

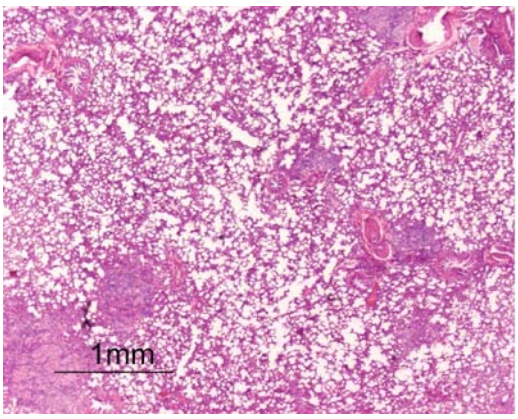


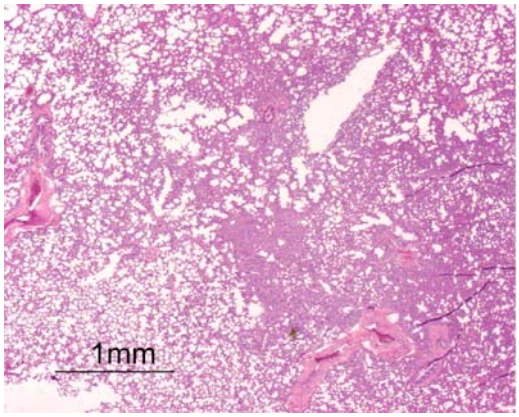
Lung Histopathology

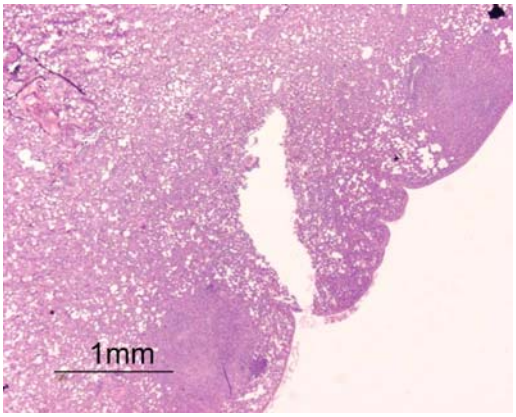
Lungs were evaluated in terms of number of granulomas, number of low power fields per section, and by a subjective evaluation score (1-4) of the condition of the lung considering the number and size of the granulomas as well as the extent of inflammatory infiltration. Two sections were evaluated for each strain at each time point. The wild-type strain had the most lesions and highest score at week 3 but by week 6 the scores were rather similar for all 3 strains. Portions of lungs with lesions are shown in the micrographs [20x].

Strain	**Week 3 Mean # Lesions/field**	**Week 3 Score**	**Week 6 Mean # Lesions/field**	**Week 6 Score**
H37Rv	4.6	2.5	3	2.25
H37Rv Δ*PE-PGRS51* (Rv3367)	2.25	1.75	4.75	2.75
H37Rv Δ*PTRP* (Rv0538)	0.66	2	1.23	2.5

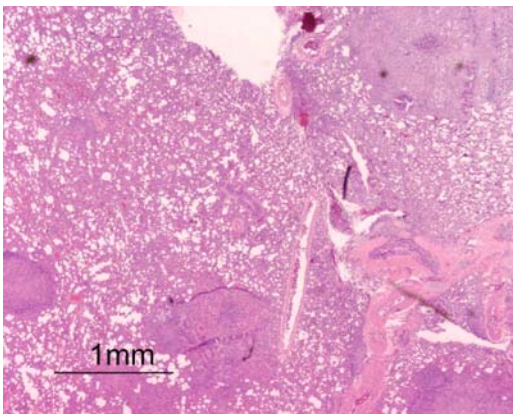
Week 3

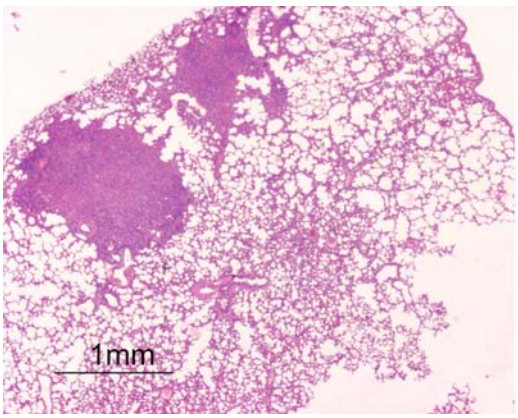


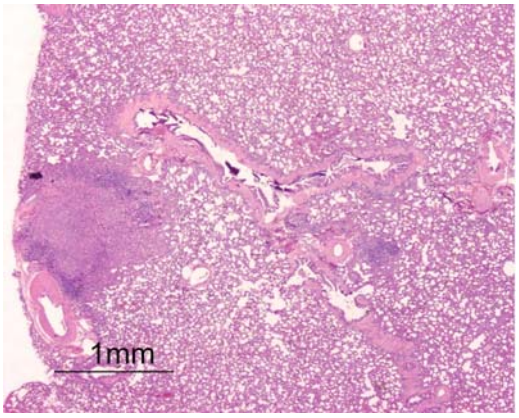




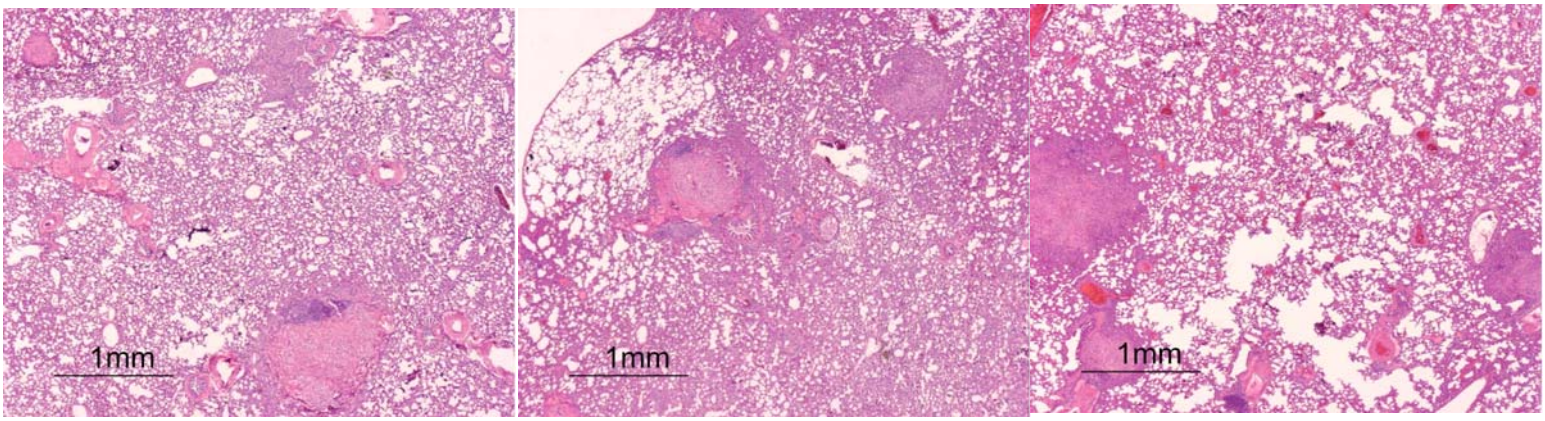
H37Rv **ΔPE-PGRS51** **ΔPTRP**







Week 6



H37Rv **ΔPE-PGRS51** **ΔPTRP**