Influenza virus exposure decreases host derived protection against *Mycobacterium tuberculosis* infection in ferrets

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Ferret as a model for TB

• Good transmission model in Flu.
• Cough
• Cheaper than NHP and cattle to procure and house within containment.
• Outbred
• Susceptible to mycobacteria- *M. bovis*, *M. avium*, *M. microti*, and *M. celatum*. 
**Study schematic**

*M.tb* infection

<table>
<thead>
<tr>
<th><strong>Dose</strong></th>
<th><strong>Time points</strong></th>
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<tbody>
<tr>
<td>Low (10-50 cfu)</td>
<td>2 wk samples</td>
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<tr>
<td>Medium (100-200 cfu)</td>
<td>4 wk euthanize</td>
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<tr>
<td>High (&gt;5000 cfu)</td>
<td>8 wk Skin test + euthanize</td>
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**Samples for ELISA**
- Serum
- Nasal wash

**Samples for MIGT & PCR**
- Nasal wash
- Gastric lavage/ stomach
- Throat swab
- Feces
- Lung
- Liver
- Spleen

**Organs for histology**
- URT
- Lungs
- MLN
- Liver
- Spleen
- MLN

**CFU**
- Lung
- Liver
- Spleen
- MLN

**End points**
- Weight change
- Temperature
- PPD reaction
- IFNγ release
- Organ bacterial load
- MIGT- nasal wash, throat swab, feces, liver, stomach, lung, spleen, trachea
- PCR
- Lung cytokine profile
M. tb infected ferrets develop positive PPD reaction
Dose dependent bacterial load in ferret tissues

**Bacterial load in ferret organs.** Ferret organ homogenates were prepared in PBS with 0.05% Tween 80. Serial dilutions were plated on Middlebrook 7H10 agar with 10 µg/ml cycloheximide. The plates were kept at 37°C for 3-6 weeks. Bacterial load in the lungs (A) reached a plateau by week 7 post infection while also (B) disseminating to the spleen, and mediastinal lymph nodes (MLN) by 8 weeks post infection (pilot study; n=3 animal per time point).
Histopathological scores of *M. tb* infected ferret tissues
Summary

- Ferrets can be infected with *M. tb*.
- Unlike guinea pigs, they are not susceptible to *M.tb* infection.
- *M. tb* infected ferrets generate a positive PPD reaction. Thus, easy diagnosis.
- Weight loss, PPD skin test, and whole blood IFNg are key correlates of infection.
- Depending upon infection dose ferrets can develop caseous necrotic granulomas in lungs or can clear/ control the infection.
- There is an exponential growth of *M.tb* in ferrets as well as dissemination to LN and spleen.
Exposure to respiratory viruses especially influenza will increase susceptibility of ferrets to *M. tuberculosis* infection.
Study schematic

**Day -7**
- Temp probe
- Nasal wash
- Blood

**Day 0**
- H3N2 i.n.

**Day 3**
- NW plaque assay

**Day 14**
- M.tb i.t.

**Day 30**
- M.tb i.t.

**Day 90**
- Euthanize

**Strains**
- A/Port Chalmers/1/1973 (H3N2)
- M. tb Erdman

**Symptoms**
- Mild weight loss
- Lethargy
- Mild nasal discharge

**Samples**
- Nasal wash
- Blood
- Tissues
- Feces

**End points**
- Weight change
- Temperature
- PPD reaction
- IFNg release
- Organ bacterial load
- NW plaque assays
- PCR
- Lung cytokine profile
Increased bacterial load in the ferrets pre-exposed to influenza
Flu infection affects humoral response against M. tb WCL
Flu infection dampens *ex vivo* IFNg release against M. tb WCL

**A) Splenocytes**

- Uninfected
- H3N2
- *M. tb*
- H3N2 + *M. tb*

**B) Whole blood**

- Uninfected
- Only *M. tb*
- H3N2 + *M. tb*
No differences in histopathological scores of ferret lungs infected with only *M.tb* vs co-infected with influenza.
Summary and Future Directions

- Preexposure to influenza cause higher bacterial load in infected ferrets. However, there was no difference in the lung inflammation score.
- *Ex vivo* stimulation of influenza pre- exposed ferret splenocytes and whole blood cells with M.tb WCL have dampened IFNg release.
- Real-time PCR of proinflammatory genes is ongoing.
- Studies with increased animal number and another with closer time point between infections are planned.
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