Murine model of nose-only small particle aerosol infection with virulent *Francisella tularensis*

Technical aspects and considerations

Presentation Outline

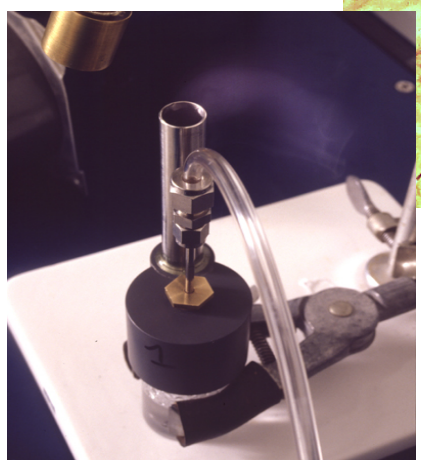
- ***Francisella tularensis***
- **In-Tox small animal nose-only aerosolizer**
- **Key steps of the aerosol procedure**
- **Mouse model of nose-only aerosol infection with virulent *F. tularensis***
- **Modifications and lessons**

Francisella tularensis

- **A gram -ve, small facultative intracellular coccobacillus**
- **A potential agent of bioterrorism (CDC Category A)**
- ***F. tularensis* subspecies *tularensis* (type A) and *F. tularensis* subspecies *holarctica* (type B)**

F. tularensis: a pathogen with potential

- Spread by ingesting contaminated food and water
- Can penetrate unbroken skin when handling infected animals
- Spread by biting insects
- Spread by aerosol



Human Tularemia

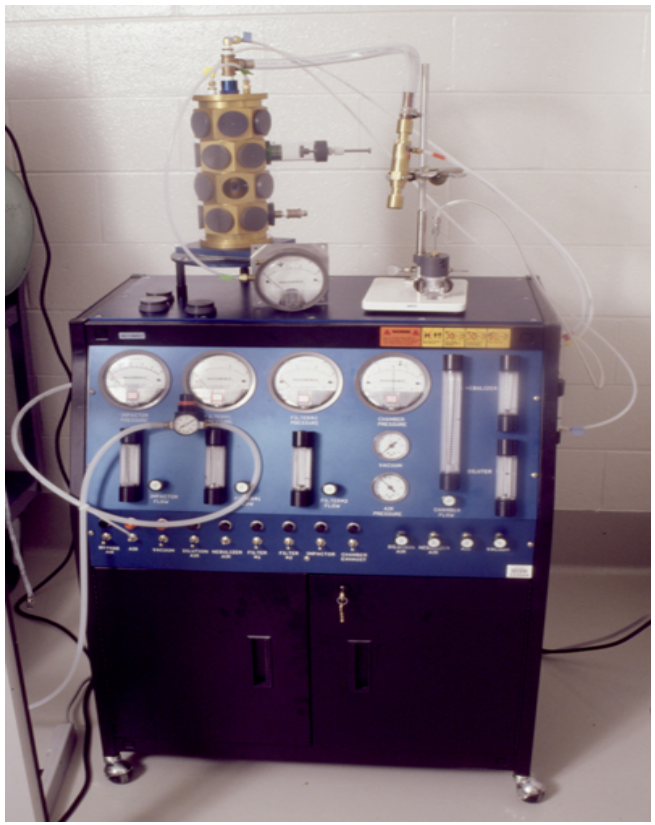
"A Rose by Any Other Name"

- **Infective dose: 10-50 organisms**
- **Incubation period: 3-5 (1-21) days**
- **Duration of illness: ~2 weeks**
- **Mortality: route of infection, bacterial strain and treatment**
- **Contagious: no**
- **Persistence of organism: months in moist soil**
- **3rd most common lab-acquired infection**
- **Vaccine efficacy: ??**

Why nose-only aerosol infection with *F. tularensis*

- Most likely mode of delivery in the event of bioterrorism
- Virulent *F. tularensis* can readily initiate systemic infection via various routes
- Generates far less aerosol
- No need for animal decontamination after the exposure

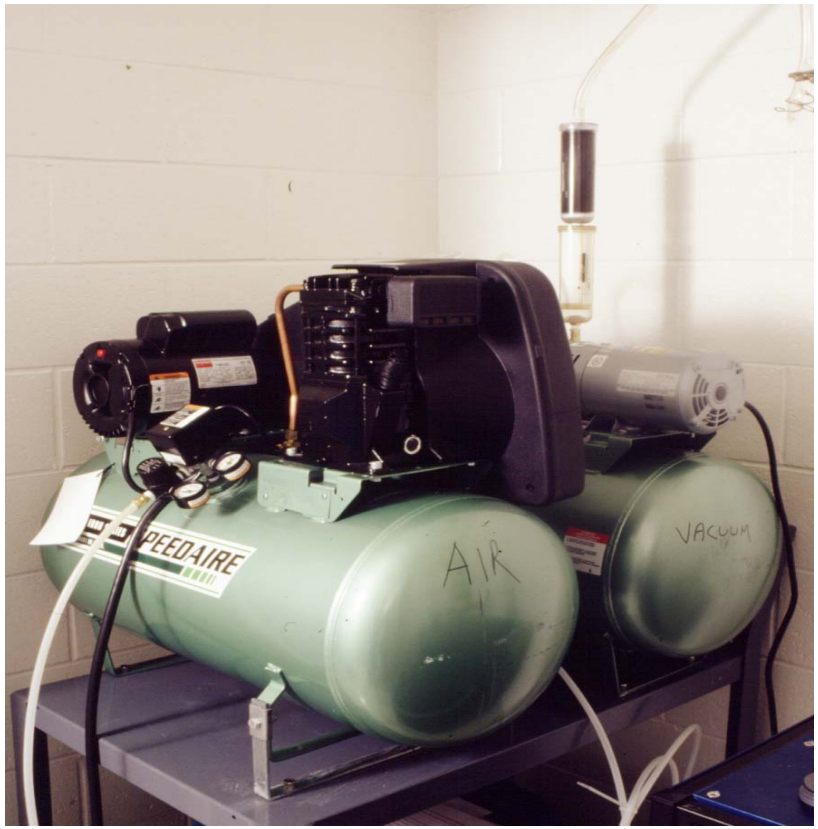
In-Tox nose-only aerosolizer



In-Tox nose only aerosolizer system

Air supply and exhaust supply subsystem

Built–in compressor and vacuum pump



- **Provide compressed air (40 psi) and vacuum to the Inhalation Exposure System**
- **Compressor is very noisy**

Air supply and exhaust supply subsystem:

HEPA-filtered building compressed air

Airflow diagram

Building compressed air

Humidified (Dilution air)

Nebulizer (nebulizer air)

Exposure chamber (Bypass air)

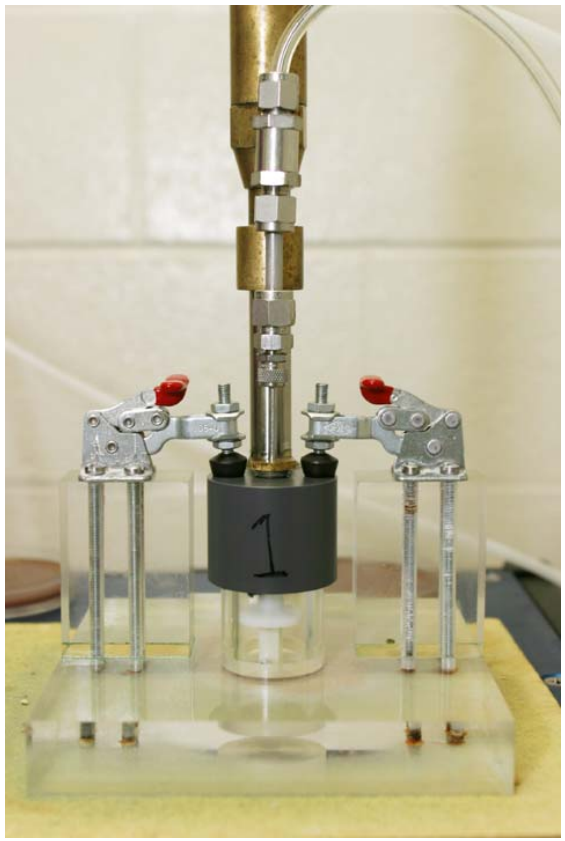
Chamber exhaust

HEPA filter 2x

Monitoring cage

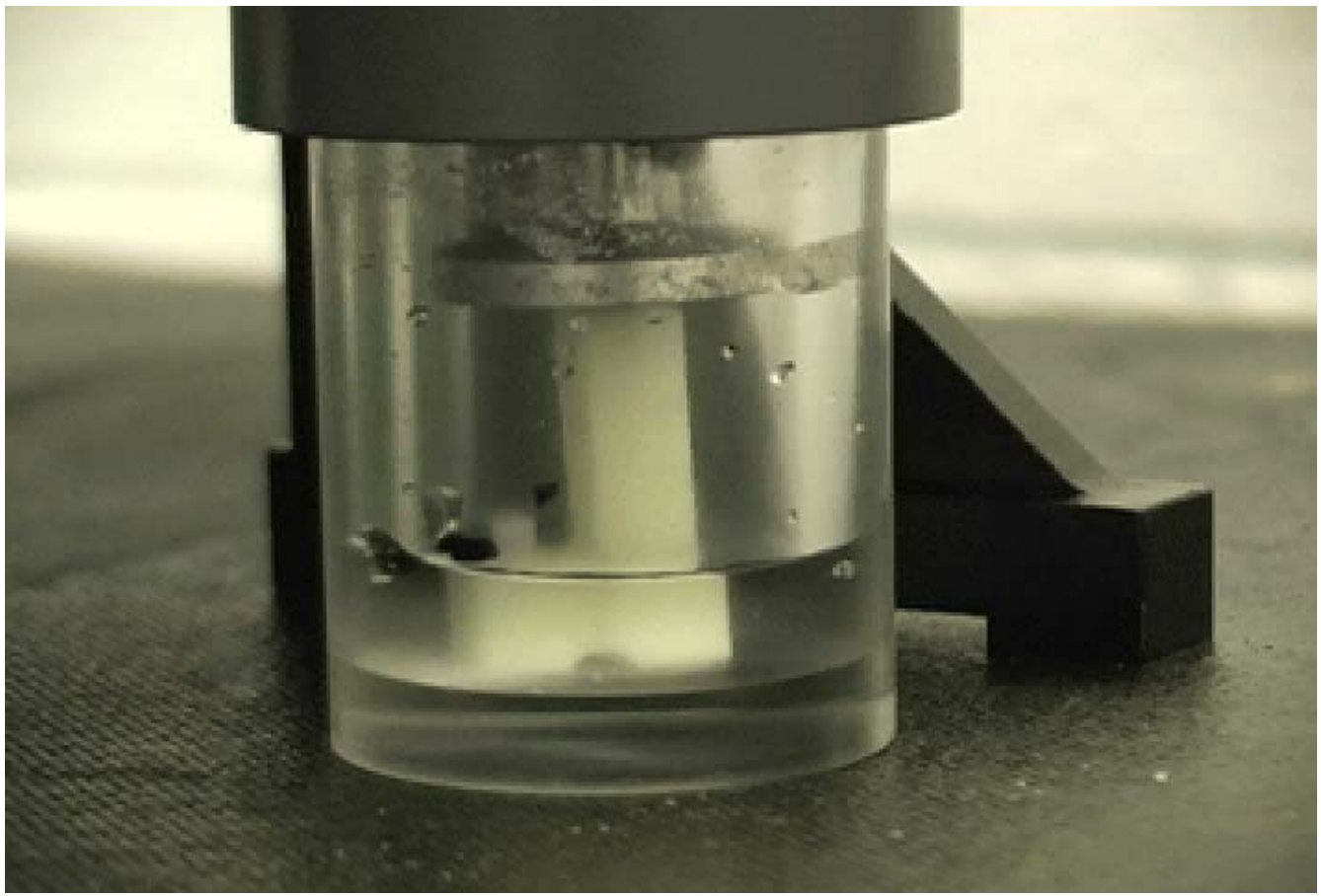
The aerosol generation subsystem:

Lovelace nebulizer

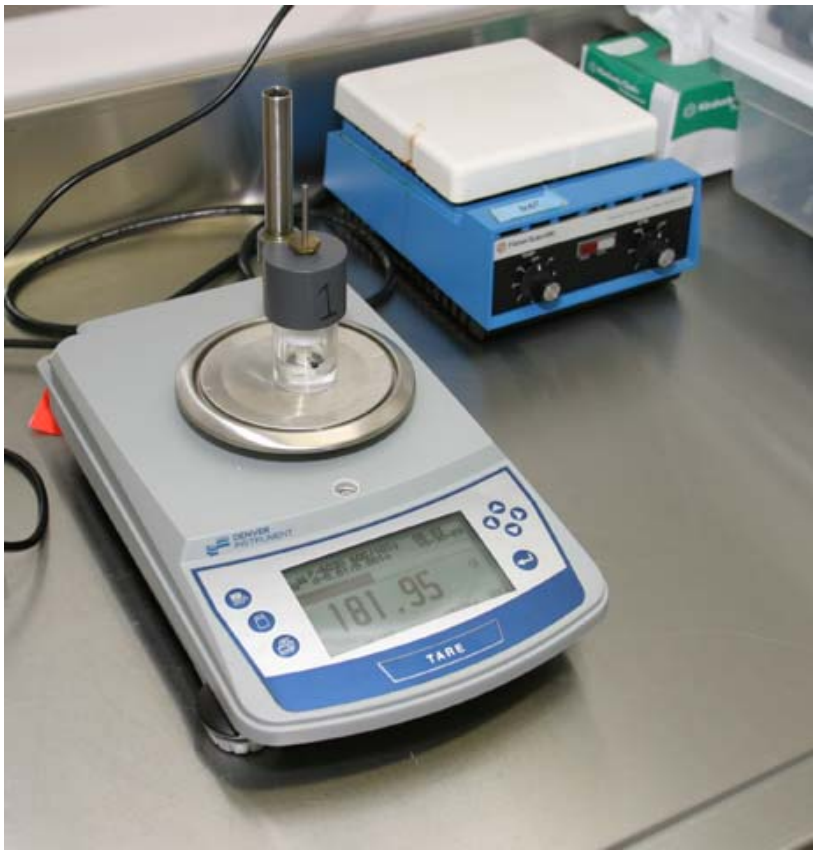


- A customized holder secures the nebulizer
- The nebulizer inlet is guarded by a check valve
- Operate at 40 psi and generate aerosol particles at 4-6 μm range
- Not measured in real time and relied on manufacturer's reassurances

Lovelace nebulizer



Lovelace nebulizer



- Consumption of the nebulizer content: 0.3 ml for a 5 min exposure
- Quantitative bacteriology on the nebulizer contents: ~50% drop in viability

The nose-only inhalation chamber

Exposure chamber

- Nebulizer connected directly to the exposure chamber
- Aerosol is diluted with fresh air
- It is fed into the exposure chamber at ~ 15L/ min

The nose-only inhalation chamber

Exposure chamber

- Chamber consists of inner and outer cylinders connected to each other by ports
- Aerosol pumped into the inner cylinder
- 24, 48 and 72 port chambers are available
- Unused ports remain capped during a run

Mouse restraining tube and mouse loading

- Radial holes aligning with nares of restrained mouse
- Plugged into a port-hole protruding from the inner exposure chamber
- The aerosol entering the inner cylinder is pulled into the outer cylinder at a slightly faster rate via the holes in the nose cone and hence across the breathing zone of the mouse

The chamber exhaust subsystem

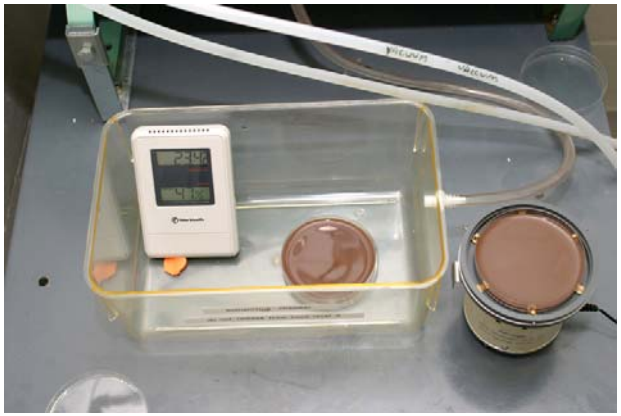
- Exhaust aerosol from the outer cylinder of exposure chamber
- Maintain a negative pressure differential between the exposure chamber and the cubicle holding the aerosolizer
- For aerosol sampling

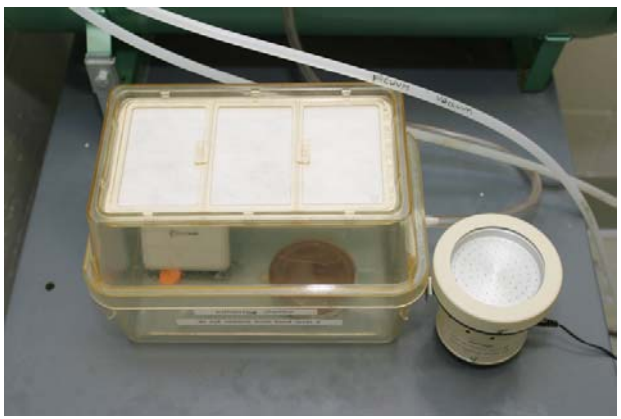
- Aerosol is exhausted via two HEPA filters connected in series
- Original apparatus supplied with charcoal filters

The chamber exhaust subsystem

- **Vacuum pump located in the cubicle for biosafety reasons**
- **Vacuum pump exhaust is guarded by a 0.22 μm absolute filter**

The chamber exhaust subsystem





- Vacuum pump exhaust is pumped across settle plates to monitor filter integrity
- An air sampler checks for direct leaks from the apparatus
- Filters replaced annually after ~ 100 runs and no filter failures ever recorded
- Air sampler detected leaking bacteria when > 10^{11} cfu/ml test samples used

Aerosol procedure

- The exposure apparatus is positioned in front of the glove ports, and the cubicle doors are closed
- The cubicle is equipped with an alarmed magnehelic gauge for negative pressure

Aerosol Procedure

Leak Test

Nebulizer test

Chamber air balancing

Aerosol exposure run

Decontamination

- During a run, three independent air streams are operating: nebulizer air, dilution air, and chamber exhaust air
- Balancing these air flows is easiest achieved by adding a magnehelic gauge coupled directly to the exposure chamber.

- Air flows into the chamber at least 15 L/min
- A negative pressure differential of 1.5 inches of water between the outer cylinder and the cubicle holding the aerosolizer

Post aerosol

- At the end of an exposure the air flowing to the nebulizer is shut off
- Two min later the dilution air is exchanged for sterile fresh bypass air
- This runs for 10 min and helps flush out any residual contaminated air

- The nebulizer cup is exchanged for one containing 10% buffered formalin
- An aerosol of formalin is sprayed through the apparatus for 30 min to decontaminate it between runs
- Run through a further 30 min with dilution air

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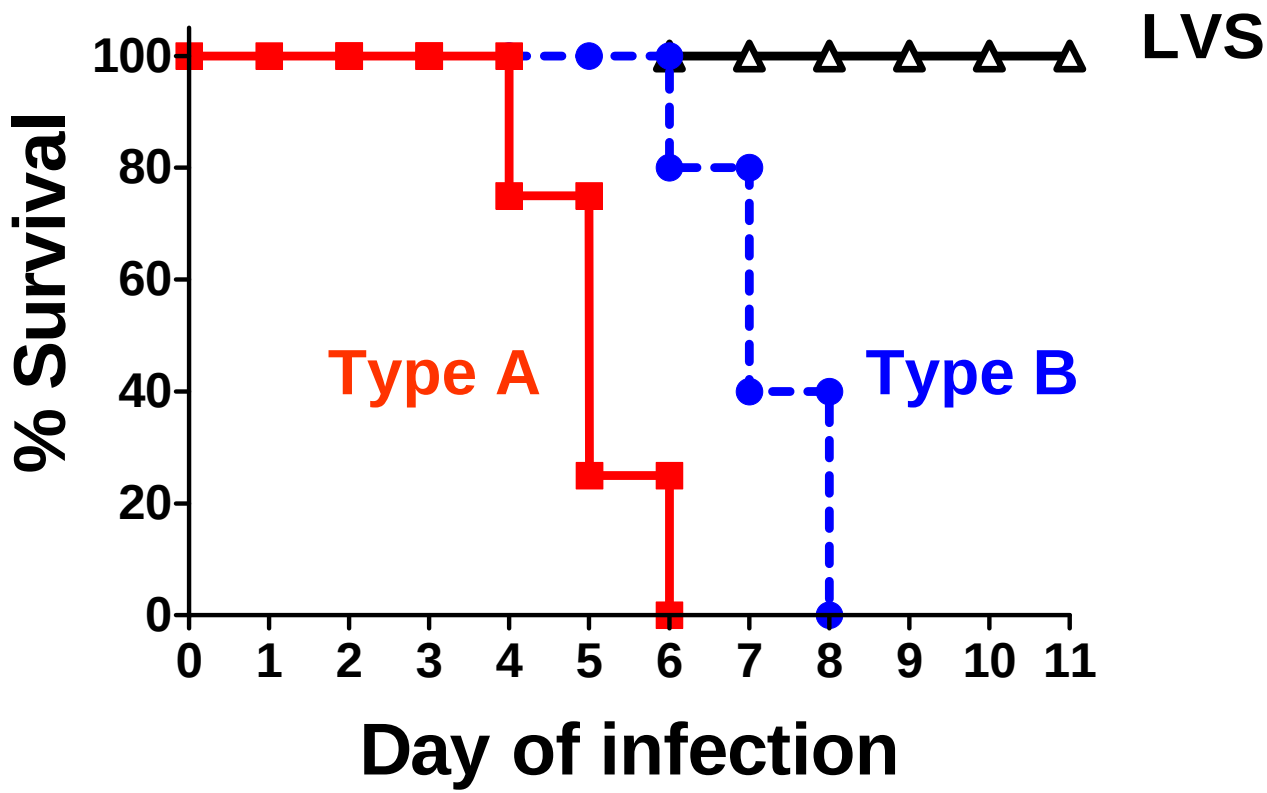
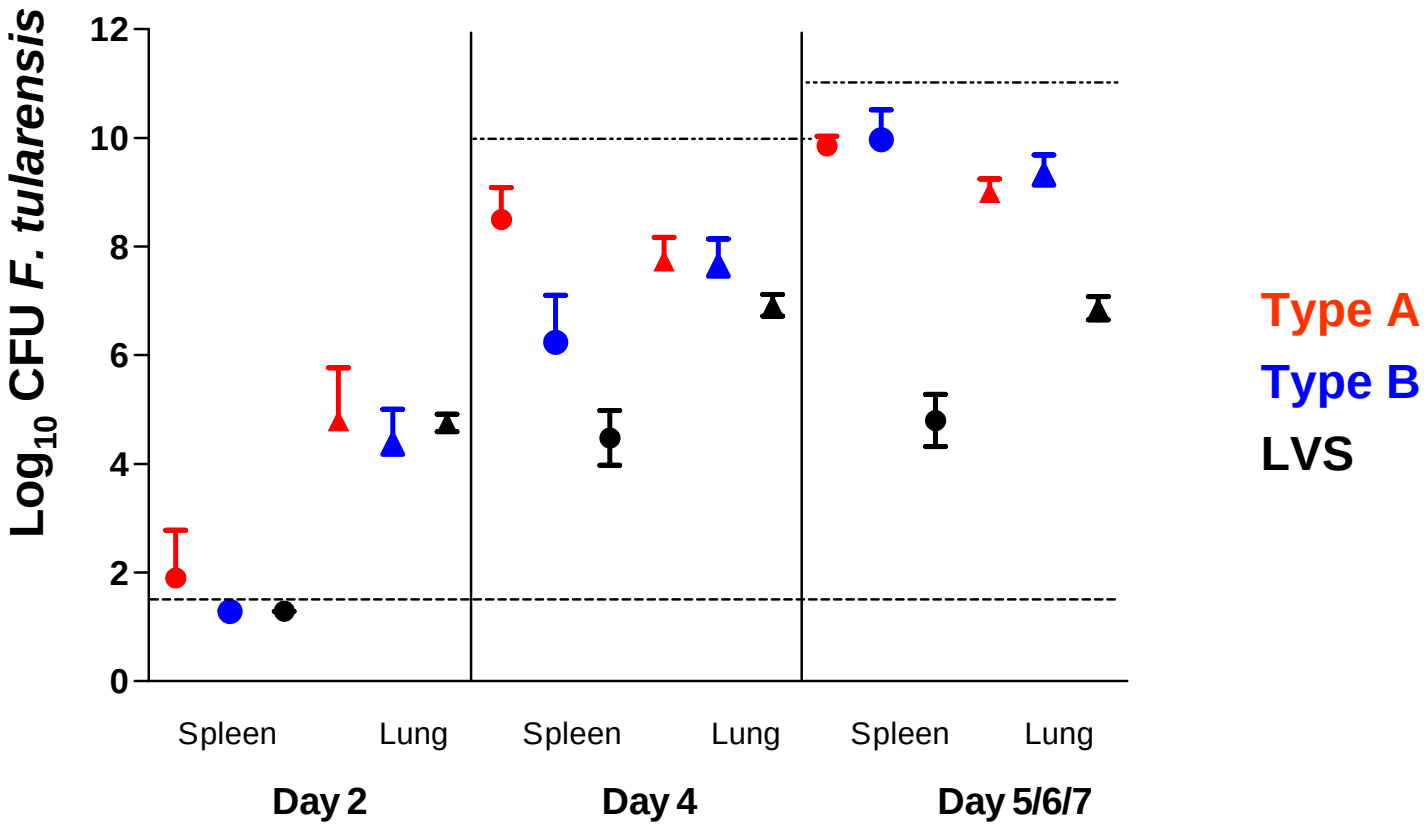
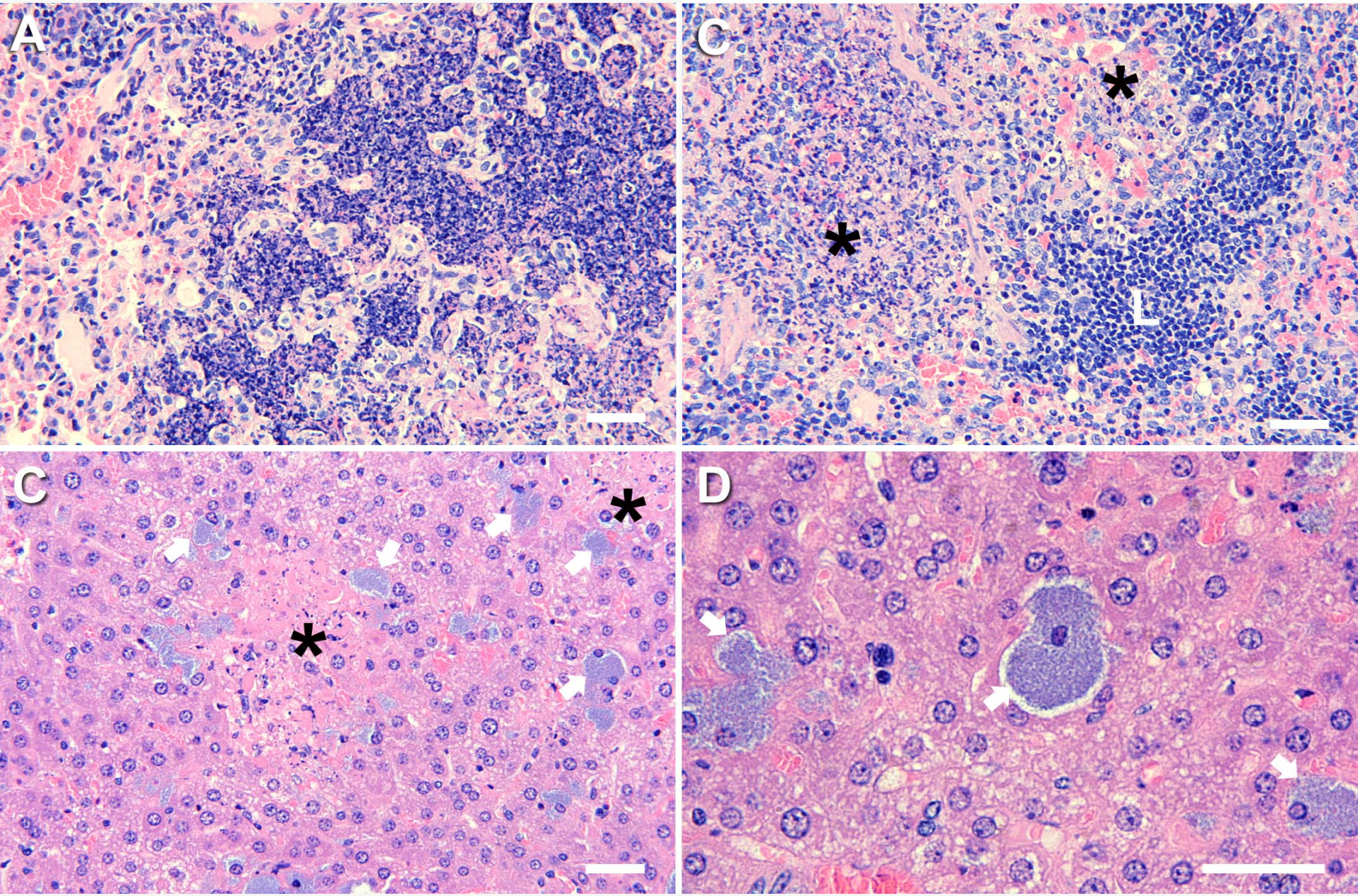
Mouse model of nose-only low dose aerosol infection with virulent F. tularensis

Aerosol infection of mice with low dose of virulent *F. tularensis* is lethal



Systemic bacterial dissemination in mice infected by aerosol with *F. tularensis*

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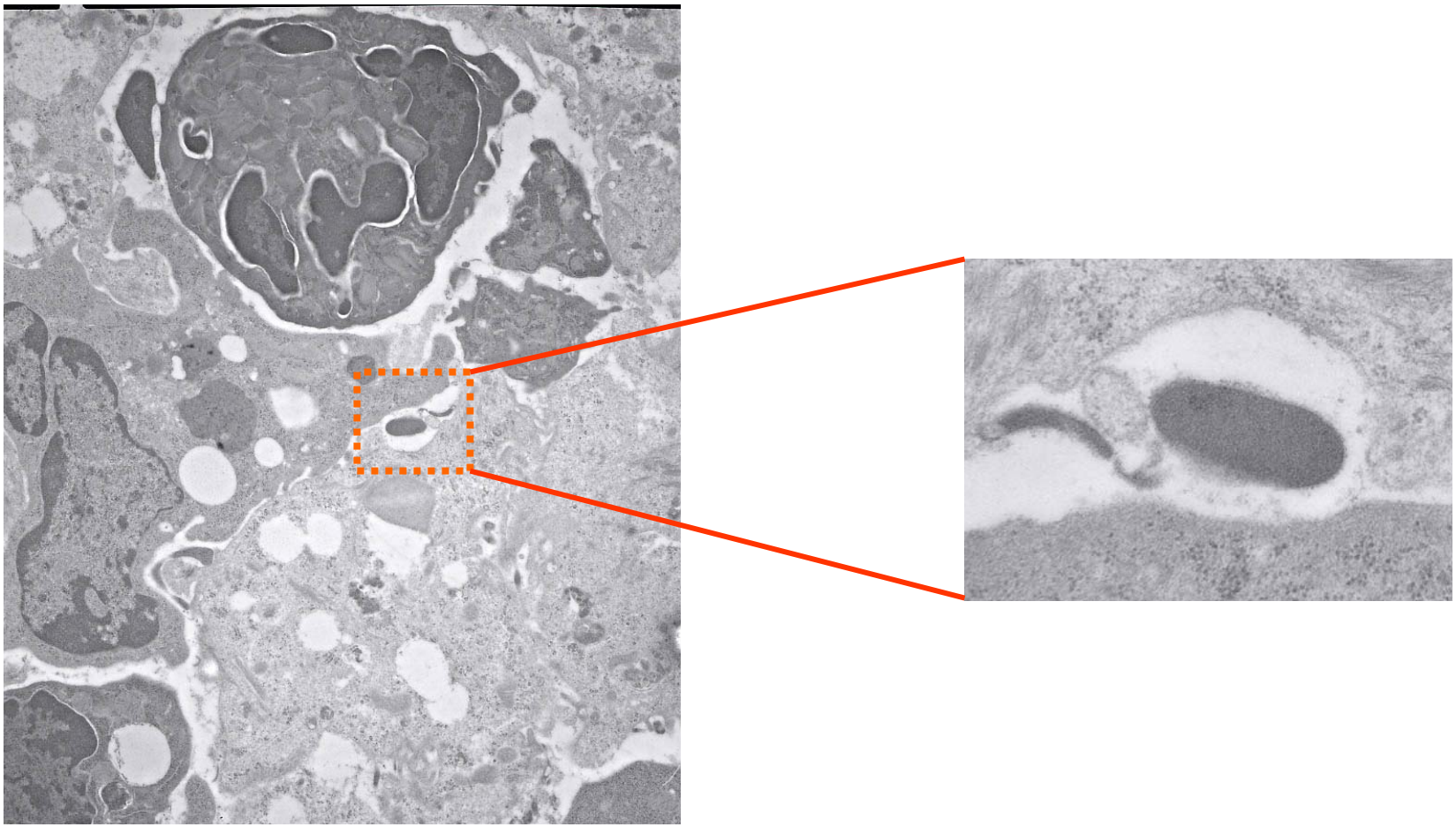


Summary

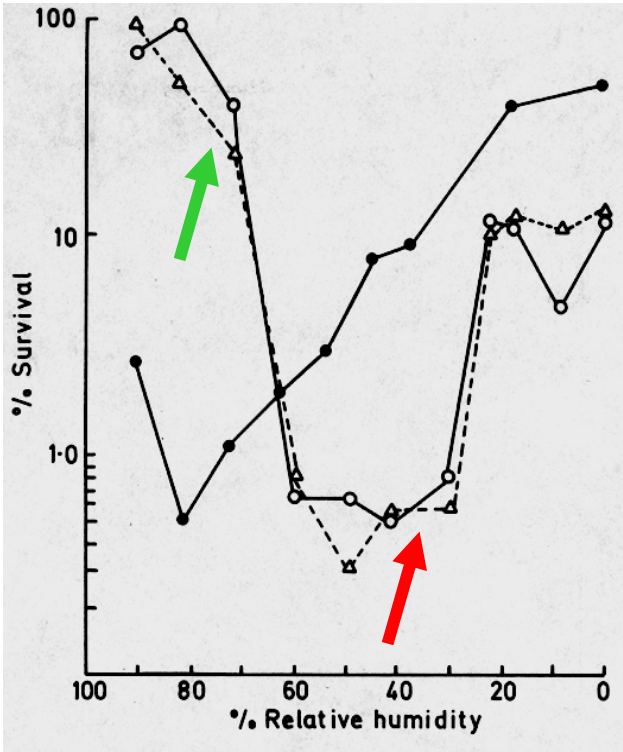
- Acute, fatal infection in mice with an LD_{50} < 10 organisms
- Rapid systemic dissemination, bacteremia, and multi-organ failures
- Severe but limited lung involvement
- Limited tissue inflammatory responses
- Extensive liver damage
- Severe and extensive lymphoid tissue damage

Modifications and lessons

- *F. tularensis loves high humidity*
- *Instrument modifications*
- *Disinfectants*

Francisella tularensis loves high humidity

- Performed initial aerosol with LVS at 40-50% RH
- No LVS recovered from the lungs 1 hr post-exposure even at high dosage
- LVS aerosolized from the wet state rapidly lost viability below 70% RH



By Cox C.S., The aerobiological pathway of microorganisms

Humidified air supply

Summary of instrument modifications

- Replace air compressor with building compressed air
- Customized nebulizer cup holder
- Replace brass coupling parts of flow meters with stainless steel parts to prevent corrosions
- Add a magnehelic gauge for monitoring all air flow rates
- Replace the charcoal filter in the chamber exhaust system with HEPA filters and fit a 0.22 μm absolute filter into the vacuum pump exhaust system

Mouse restraining tubes

- Change the galvanized metal restraining bolts with stainless steel bolts
- Sponge stoppers: easy on mice and act as additional filter for aerosol leakage into the restraining tube
- Do not load mice too tightly into the restraining tube

Disinfectants

- *F. tularensis* is very susceptible to 0.1% Quatsyl, household bleach and 70% ethanol
- Bleach is too corrosive to the nose-cones and restraining bolts
- Ethanol destroys the various O-rings on the restrainer and disintegrates Perspex components
- Remove all traces of Quatsyl

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