



**UNITED STATES ARMY MEDICAL RESEARCH
INSTITUTE OF INFECTIOUS DISEASES**

*Bio*defense solutions to protect our nation

**Aerosol Monitoring of ABSL-3 Suites
Housing Non-Human Primates
Challenged with *Coxiella burnetii***

Dr. David Harbourt
October 15, 2018



Disclaimer

*Bio*defense solutions to protect our nation

The views, opinions and findings contained herein are those of the author and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.



IACUC Disclaimer

Biodefense solutions to protect our nation

Research was conducted under an IACUC approved protocol in compliance with the Animal Welfare Act, PHS Policy, and other Federal statutes and regulations relating to animals and experiments involving animals. The facility where this research was conducted is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011.



Outline

Biodefense solutions to protect our nation

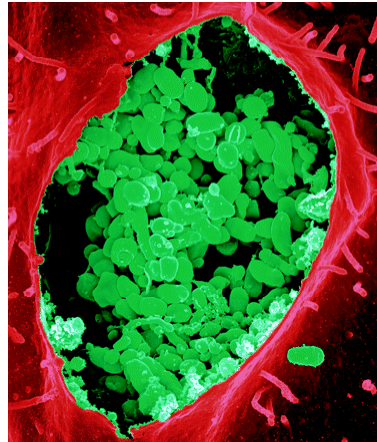
- Background on *C. burnetii*
- Proof of Concept/Purpose
- Methodology of DNA sampling and extraction
- Results of air sampling studies in animal rooms
- Recommendations/Findings
- Study and regulatory hurdles



Background

Biodefense solutions to protect our nation

- *Coxiella burnetii* is an obligate intracellular gram negative bacteria with worldwide distribution
- Second most common laboratory acquired infection
- Very low infectious dose and is resistant to common bacterial disinfectants
- Most animal model studies have focused on mice
- NHP studies previously carried out at USAMRIID, new studies attempted to establish appropriate challenge dose for countermeasure development



High Volume Air Sampler

Biodefense solutions to protect our nation

- Dry Filter Unit 1000 (DFU 1000) used for all animal room studies
- 100 cfm sampling rate
- Able to sample continuously for extended periods
- Durable and water resistant
- Utilized membrane (<math><1\mu\text{m}</math>) and standard $1\mu\text{m}$ filters to capture particulates



Air Inlet

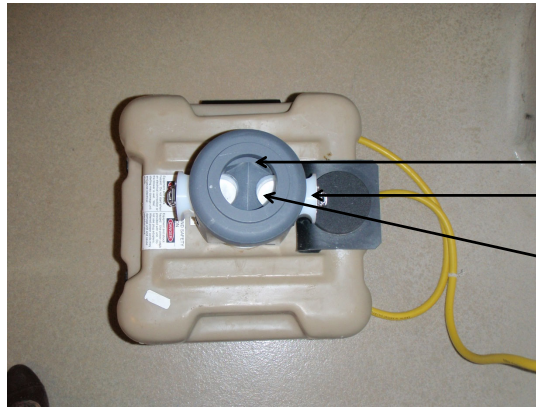
Filter housing

Exhaust



High Volume Air Sampler (Top Down View)

Biodefense solutions to protect our nation



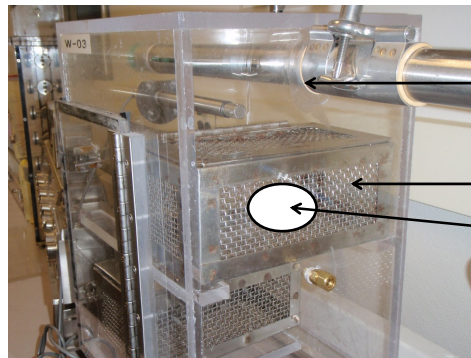
Air Inlet
Filter housing
Filter



Proof of Concept Study

Biodefense solutions to protect our nation

- Collection occurred during NHP challenge spray conducted inside Class III BSC
- *C. burnetii* spray concentration ~1000 GE/mL
- Filters placed within exposure chamber to examine potential DNA recovery
- Following spray filters were placed into media for extraction and incubation prior to PCR analysis

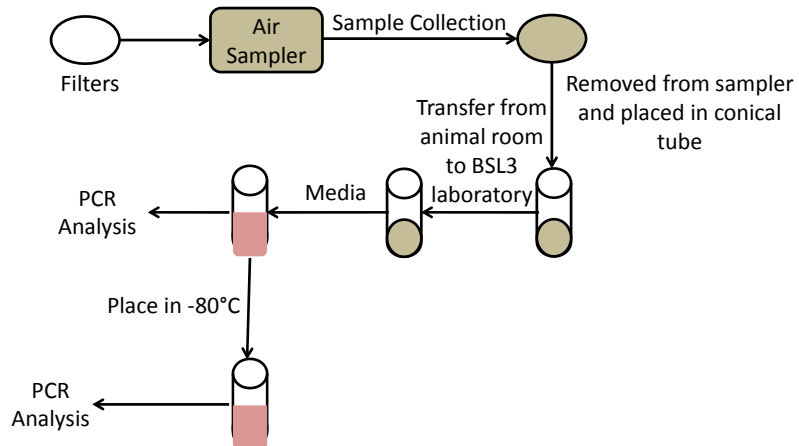


Collision tube
(Aerosol source)
Aerosol Chamber
Air Sampler
Filters



Filter Extraction Methodology

Biodefense solutions to protect our nation



Animal Study Methodology

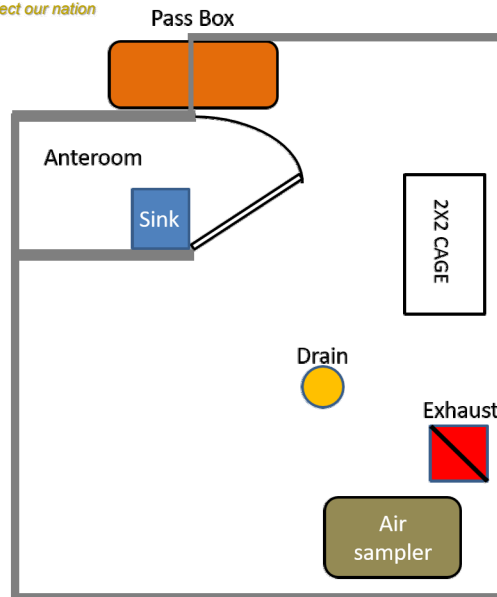
Biodefense solutions to protect our nation

- Sampling conducted during two independent animal studies
- Studies were carried out in different ABSL3 suites by different research groups
- Sampling was carried out throughout the duration of each study
- NHPs were challenged with *Coxiella burnetii* at different dose levels
- In each study, air samples as well as fecal and urine samples were collected to attempt to correlate shedding with air sample results



Study One Part One Setup

Biodefense solutions to protect our nation



Study Complications

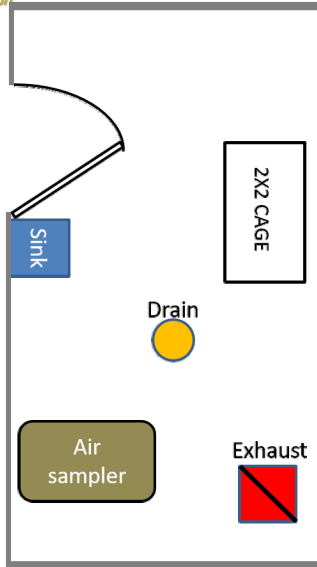
Biodefense solutions to protect our nation

- A water leak started in the floor above the animal room due to a leaking autoclave condensate pipe
- Study had to be transferred to another room inside the same ABSL-3 facility
- Room had to be suitable for *C. burnetii* work along with telemetry cabling requirements



Study One Part Two Setup

Biodefense solutions to protect our nation



Study One NHP Outcomes

Biodefense solutions to protect our nation

Route – Aerosol challenge (head only)

Dose – 1×10^7 GE/mL (*C. burnetii*)

NHP type – Cynomolgous macaque

Outcome (in terms of survival) – Both NHPs survived through the end of the study (28 days)



Study One Results

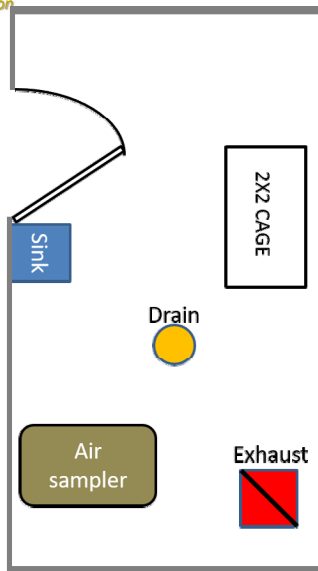
Biodefense solutions to protect our nation

Day	Air Sample	Urine	Feces
0	Negative	Negative	Negative
1	Negative	Negative	Negative
2	Negative	Positive	Negative
5	Negative	Negative	Negative
6	Negative	Negative	Negative
7	Negative	Negative	Negative
8	Negative	Negative	Negative
9	Negative	Negative	Positive
12	Negative	Negative	Positive
15	Negative	Negative	Positive
21	Negative	Negative	Negative
28	Negative	Negative	Negative
Positive Control 1	Positive		
Positive Control 2	Positive		
Negative Control	Negative		



Study Two Setup

Biodefense solutions to protect our nation





Study Two NHP Outcome

*Bio*defense solutions to protect our nation

Route – Aerosol challenge (head only)

Dose – 3×10^8 GE/mL (*C. burnetii*)

NHP type – Cynomolgous macaque

Outcome (in terms of survival) – NHP suddenly became unresponsive with labored breathing on day 7 and was euthanized



Study Two Results

*Bio*defense solutions to protect our nation

Day	Air Sample	Urine	Feces
0	Negative	Negative	Negative
1	Negative	Negative	Negative
2	Positive	Negative	Positive
5	Negative	Positive	Negative
6	Positive	Negative	Positive
7	Negative	Negative	Positive
Positive Control 1	Positive		
Positive Control 2	Positive		
Negative Control	Negative		

Note: Study was terminated after animal was euthanized on day 7



Difficulties

Biodefense solutions to protect our nation

- ABSL-3 entry/exit and specimen transfer
- Maintenance schedule for BSL-3 laboratory suites
- IACUC approval conditions for animal studies
- New FDA Well Documented Study requirements



Discussion

Biodefense solutions to protect our nation

- PCR analysis conducted using specific markers for *C. burnetii* during both proof of concept and sampling studies
- Proof of concept demonstrated successful bacterial DNA recovery through PCR in each replicate
- Placement of high volume air samplers was limited due to space and safety considerations
- Membrane filters must be used in conjunction with standard filters to prevent degradation



Discussion

Biodefense solutions to protect our nation

- Positive PCR results obtained during peak stages of infection in Study 2
- Positive PCR results obtained in both urine and feces samples in both Study 1 and Study 2
- Limited by the numbers of NHPs in both studies prevent conclusive analysis
- Hallway sampling was not conducted during either study due to safety considerations
- While aerosolization of *C. burnetii* DNA may be observed during peak periods of infection it is an infrequent occurrence



Conclusions/Recommendations

Biodefense solutions to protect our nation

- *C. burnetii* DNA can be shed by NHPs both early and peak stages of infection
- Potential presence of aerosolized *C. burnetii* occurs infrequently and is study dependent
- Results of current study consistent with what was seen in EBOV sampling studies
- NHP outcomes related to *C. burnetii* challenges are dose dependent
- PPE and engineering control recommendations need to be evaluated on a case by case basis



Acknowledgements

Biodefense solutions to protect our nation

- Dr. Sara Ruiz
- Christopher Jensen
- Bill Dorman
- Samantha Tostensen



Questions?

Biodefense solutions to protect our nation