Development of an Aerosol Delivery System for Multiple Simultaneous Exposures of Rabbits to Aerosolized *Bacillus anthracis*

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ABSTRACT

Objective: An aerosol model of infection for *Bacillus anthracis* Ames (BA) was developed to allow multiple simultaneous aerosol exposures of New Zealand White (NZW) rabbits using a modified 64 port inhalation exposure chamber with nebulizers. This development allows for aerosol exposure to multiple rabbits which minimizes the number of runs needed and reduces the variation of exposure concentration for the study.

Methods: BA spores were prepared using the growth parameters of Leighton and Doi (1971). Titer was determined using Trypticase Soy Agar. For the determination of LD_{50} and spray factor, presented challenge dose concentrations of 0, 10^4, 10^5, 10^6, and 10^7 CFU/mL were prepared to generate a respirable aerosol that was supplied to a modified 64-port nose-only inhalation chamber. Three NZW rabbits were exposed per BA concentration tested. The inhalation chamber was modified for up to eight NZW rabbits for nose only exposure. With 52 ports blocked, remaining ports were enlarged to ½" dia. for increased flow with 8 ports for rabbits and 4 ports for monitoring (AGI and viable impactor).Four Pari LC Plus nebulizers operated simultaneously at 28 PSI.

Results: The spray factor, calculated as a ratio of exposure chamber aerosol concentrations to starting concentration, ranged between 7.45E-06 to 2.16E-05 for the concentrations of BA spores tested for an average of 1.28E-05 +/- 7.26E-06. Particle size was between 1-3 mm MMAD. The LD_{50} in NZW rabbits was 4.19E05 CFU/animal.

Conclusions: Results indicate that this developing novel bioaerosol exposure platform allows for multiple simultaneous and consistent exposures of at least three NZW rabbits including peripheral monitoring apparatus. While further testing is needed to test the consistency of exposure with up to eight animals/run, this platform has the capability of consistent delivery of aerosol to each port, thereby reducing variation in aerosol concentration and the number of runs needed for a therapeutic or vaccine study.

INTRODUCTION

Bacillus anthracis, a Gram-positive bacterium with the capability to form endospores, is the causative agent for inhalational anthrax. Additionally, *B. anthracis* is a potential agent to be used for bioterrorism purposes, especially by release as an aerosol. Despite a more rapid onset of disease, disease progression in rabbits (New Zealand White-NZW), appears to be similar to human progression, thereby indicating that the NZW rabbits are an appropriate model for evaluation of therapeutics and vaccines (Yee, et al. 2010).

Our goals for this study were three-fold: 1) to develop and characterize a New Zealand White rabbit model for inhalational anthrax using an IITRI-developed, nose-only bioaerosolization system; 2) to determine if this system could challenge multiple animals at once, and 3) to determine the spray factor/operating characteristics of this system as well as the aerosol LD_{50}.

METHODS

***B. anthracis* Challenge Material preparation:** For preparation of *B. anthracis* spores, A sublot of *B. anthracis* Ames strain spores, stored at 2-8° C was isostreaked with a sample from the main stock. Well isolated colonies were selected and inoculated into baffled-bottomed, vented, glass flasks containin Leighton-Doi Broth. The innoculated flasks were incubated at 37±2°C for ~96 hours at approximately 175 rpm. After incubation the broth was centrifuged, supernatant was removed from the *B. anthracis* pellet. The *B. anthracis* pellet was suspended in sterile water for injection (SWFI) and plated to determine the sub lot titer and purity. From this sub lot, the culture was prepared for aerosol exposure by dilution with SWFi to achieve the desired concentration necessary for the aerosol runs.

B. anthracis Aerosol Generation: The culture was serially diluted to desired concentrations in SWFI for inhalation exposure to minimize foaming during the aerosolization process. *B. anthracis* aerosols were generated using the IITRI bioaerosol system which utilizes a 64-port, flow-past, nose-only inhalation exposure chamber (Lab Products Inc., Seaford, DE) that was modified to have 12 ports for up to 8 rabbits, all glass impinger (AGI), pressure monitoring, and Cascade impactor (for particle sizing). Nose-only rabbit restraint tubes (with attached pneumotachographs) were supplied by CH Technologies. Four Pari LC Plus jet nebulizers (Pari, Germany) were used with 20 PSI of pressurized air. Each nebulizer has a flow rate of approximately 5 LPM for a cumulative total of approximately 28 LPM. All exhaust air was passed through a series of HEPA filters prior to exiting the facility. Viable aerosol material from the breathing zone of the exposure chamber was determined by two AGIs connected in series with each containing 10 mL of sterile water, sampling at 2 LPM. Additionally, 3 mm glass beads were included in the upstream impinger to improve collection efficiency. Aerosol particle size distribution was measured by a viable 6-stage Andersen cascade impactor (Tisch Environmental Inc. OH). Trypticase Soy Agar (TSA) plates were used as the collection medium in the Andersen cascade impactor. Time of exposure for a typical run was 10 minutes . All titers of *B. anthracis* were determined on TSA and incubated at 37° C ± 2° C for approximately 18-24 hours. Pre-challenge measurements of the tidal volume and respiration of each rabbit was performed using the Buxco (Wilmington, NC) BioSystem XA (version 2.7.3) program. Analog outputs of the flow signal from the transducers attached to the pulmonary tubes were digitized on a computer with the BioSystem XA program. The software displayed the digitized waveform in real time and the calculated tidal breathing parameters (rate, minute volume, and tidal volume) during testing. Buxco has been purchased by Data Sciences International, Minneapolis, MN.

Rabbits and LD_{50} Determination: New Zealand white rabbits were used for the LD50 determination. These animals weighed on average 2.56 to 3.5 kg at the time of testing. Each experimental group had 3 rabbits. Prior to aerosol exposure to *B. anthracis*, respiration rates for the rabbits were measured via plethysmography using the Buxco BioSystem XA software. Respiration minute volume was used to determine overall dose of the rabbit upon completion of the aerosol exposure. Post-challenge, the rabbits were observed three times a day for 8 days for mortality and clinical scoring.

FIGURE 1. Modification of the 64-Port Aerosol Exposure Chamber for Use With Up to Eight NZW Rabbits

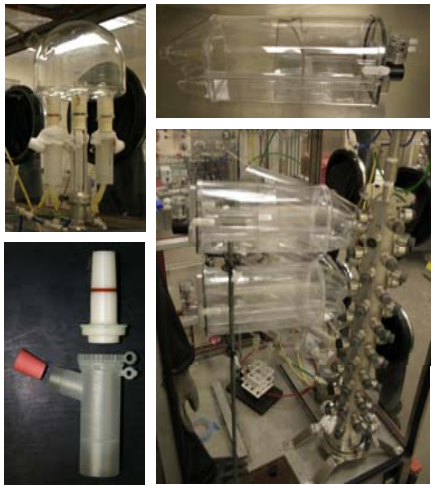


TABLE 1. Summary of Aerosol Exposure Parameters for LD50 Determination in NZW Rabbits

		Group 1	Group 2	Group 3	Group 4	Group 5
***B. anthracis* Concentration**	*Starting Concentration (CFU/mL)*	0	$1.1x10^6$	$1.3x10^7$	$1.2x10^8$	$1.2x10^9$
	Post-Challenge Titer (AGI: CFU/mL)	0	$7.1x10^3$	$1.3x10^5$	$1.3x10^6$	$2.5x10^7$
	Presented Dose (CFU/animal)	0	$8.8x10^4$	$2.0x10^6$	$2.6x10^7$	$5.7x10^8$
Particle Size	**Particle diameter, (µm)**	NA	1-3 µm			
Spray Factor (Average 4.4 x 10^{-5})		NA	$6.3x10^{-6}$	$7.5x10^{-6}$	$1.6x10^{-5}$	$2.2x10^{-5}$

TABLE 2. Summary of Mean Time to Death and Survival for LD50 Determination In NZW Rabbits

		Females	
Group	**Actual Challenge (CFU/animal)**	**MTD (Days)**	**Survival (%)**
1	0	0	100
2	8.8E+04	0	100
3	2.0E+06	2	0
4	2.6E+07	3	0
5	5.7E+08	2	0

The calculated LD_{50} (Reed-Muench) was determined to be 4.19E+05 CFU/animal

FIGURE 2. Spray Factor Across Different Starting Concentrations of *B. Anthracis*

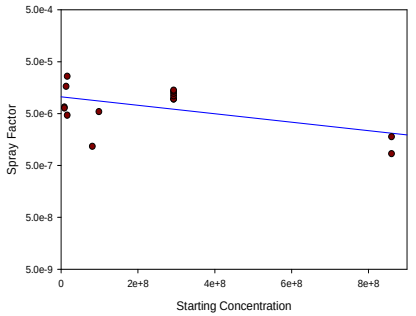
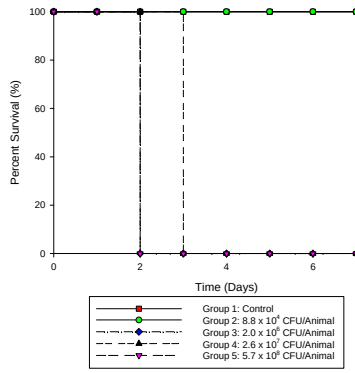


FIGURE 3. Survival of NZW Rabbits at Different Exposure Concentrations of *B. Anthracis*



RESULTS AND CONCLUSIONS

- A multiport nose-only exposure system was designed for up to 8 rabbits simultaneously exposed to *B. anthracis*. However for this test, 3 rabbits were exposed to each aerosolized *B. anthracis* concentration.
- The average spray factor of this system was 4.4 x 10^{-5}.
- The spray factor was relatively consistent through different dose concentrations (12 determinations).
- Using New Zealand White rabbits (approx 2.5 kg), the LD_{50} was calculated to be 4.19 x 10^5 CFU/animal with *B. anthracis* as the challenge agent.
- Although real-time plethysmography was not used in this test, it will be a future development for this system.
- Since the development of this multiport rabbit aerosol exposure apparatus, we have successfully tested the exposure of up to 6 rabbits simultaneously at different concentrations of *B. anthracis*. Further testing will be necessary when increasing to 8 animals challenged simultaneously.
- We believe, through our current testing, that this platform has the capability of consistent delivery of aerosol to each port thereby reducing variation in aerosol concentration and the number of runs needed for a therapeutic or vaccine study.

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