Development of *Yersinia pestis* CO92 Aerosol Post-exposure Prophylaxis and Delayed Treatment Models Using a 10-day Ciprofloxacin and Doxycycline Dosing Schedule

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ABSTRACT

Objective: A therapeutic aerosol model of infection for *Yersinia pestis* CO92 was developed to evaluate therapeutic post-exposure prophylaxis treatment (PEP) and delayed treatment strategies, with both using 10-day ciprofloxacin and doxycycline dosing schedules. The relapse potential after antibiotic treatment was also evaluated during a 14 day post-dosing recovery evaluation period.

Results: The inhaled dose was $1.76x10^5$ CFU/mouse (approximately 50 x LD_{50}). For PEP therapy groups, 100% and 90% survival was observed with ciprofloxacin or doxycycline dosed mice, respectively, during the 10 day dosing period. After completion of the PEP recovery evaluation period (day 24), 100% and 80% survival of the ciprofloxacin and doxycycline dosed mice was observed, respectively. For the delayed treatment groups (dosing start was 42 hours post-challenge), survival was 80% and 90% for ciprofloxacin or doxycycline, respectively, at the end of the dosing period. After the completion of the delayed treatment recovery evaluation period (day 26), survival was 70% and 90% for ciprofloxacin and doxycycline, respectively,. The saline control group had survival of 10% due to one control mouse surviving beyond day 4.

Conclusions: Both ciprofloxacin and doxycycline demonstrated good protection for mice infected with aerosolized *Y. pestis*. Ciprofloxacin demonstrated complete protection through the recovery phase for the PEP groups but dropped in effectiveness by the conclusion of the delayed treatment groups to 70% survival. Doxycycline showed relatively consistent effectiveness between the PEP groups (80% survival) and delayed treatment groups (90% survival).

INTRODUCTION

Yersinia pestis, a Gram-negative bacterium, is the causative agent for bubonic and pneumonic plague. Additionally, *Y. pestis* is a potential agent to be used for bioterrorism purposes especially by release as an aerosol. Pneumonic plague symptoms, when administered via the aerosol route, are generally observed in mice within 24 hours (Agar et al, Microbiology, 2008) and IITRI's historical data indicate that untreated mice generally succumb to pneumonic infection within 3-4 days. *Y. pestis* is considered a potential bioterrorism agent if released via an aerosol.

Our goals for this study were three-fold: 1) to develop and characterize a BALB/c murine model for pneumonic plague using an IITRI-developed, nose-only bioaerosolization system; 2) to determine the aerosol LD_{50} of a *Y. pestis CO92* using BALB/c mice; and 3) to evaluate and compare the efficacy of the bactericidal ciprofloxacin and the bacteriostatic doxycycline antibiotic on BALB/c mice exposed to a 50-LD_{50} aerosol challenge to *Y. pestis* for both post-exposure prophylaxis and delayed treatment models.

METHODS

Y. pestis Challenge Material: *Y. pestis* CO92 (NR-641) was obtained from *bei* resources. Frozen working cell bank *Y. pestis* stocks, stored at ≤ -65°C, were thawed and 100 µL of the culture was inoculated into 100 mL Heart Infusion broth + 1% xylose (HIBx). The culture was grown with at 28°C ± 2°C with shaking at approximately 200 rpm for 15 hours until an optical density of 1.0 ± 0.05 at 600 nm (OD_{600}) was achieved (approximately 2.0 x 10^8 CFU/mL). Purity was assessed by colony morphology and various media (Tryptic Soy Agar with 5% Sheeps Blood (TSAB), MacConkey Agar (MAC), Phenylethyl Alcohol Agar (PEA), and Cefsulodin-Irgasan-Novobiocin Agar (CIN).

Y. Pestis Aerosol Generation: The culture was serially diluted to desired concentrations in 1X phosphate-buffered saline (PBS) for inhalation exposure to minimize foaming during the aerosolization process. *Y. pestis* 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) and six Pari LC Plus jet nebulizers (Pari, Germany). With 20 PSI of pressurized air, each nebulizer has a flow rate of approximately 5 LPM each with a cumulative total of approximately 30 LPM flow rate with all six nebulizers operating concurrently. 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 all glass impingers (AGI) connected in series with each containing 10 mL of phosphate buffered saline solution. 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). Congo Red agar (CRA) 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 *Y. pestis* were determined on CRA and incubated at 28°C ± 2°C for approximately 48 hours.

LD_{50} Determination: Animals used were 9 week old female BALB/c mice weighing on average 18g to 20g. Each experimental group had 10 mice.

Antibiotic Administration: Ciprofloxacin (50 mg/kg) or doxycycline (40 mg/kg) were administered four times a day via the intraperitoneal dosing route. Dosing schedule was initiated 24 hours after exposure for the post exposure prophylaxis assessment or 42 hours after exposure for the delayed treatment assessment. After completion of the dosing phase, animals were monitored for an additional 14 days each.

RESULTS

Table 1. Summary of Aerosol Exposure Parameters for LD_{50} Determination in BALB/c Mice

		Group 1	Group 2	Group 3	Group 4	Group 5
Y. pestis CO92 Concentration	Post-challenge Titer (CFU/ml)	0	$6.4x10^4$	$6.8x10^5$	$1.2x10^6$	$3.8x10^6$
	Presented Dose (CFU/animal)	0	$1.6x10^2$	$4.7x10^3$	$1.0x10^4$	$2.0x10^4$
Particle Size	Particle Diameter µm	NA	1-3 µm			
Spray Factor (Average $3.0x10^5$)		NA	$1.3x10^{-5}$	$3.5x10^{-5}$	$4.4x10^{-5}$	$2.8x10^{-5}$

Table 2. Summary of Mean Time to Death and Survival for LD_{50} Determination in BALB/c Mice

Group	Actual Challenge (CFU/animal)	Females	
		MTD (Days)	Survival (%)
1	0	ND	100
2	160	ND	100
3	4,700	4.5	60
4	10,000	4.0	50
5	20,000	3.7	10

The calculated LD50 (Reed-Muench) was determined to be 8060 CFU/animal. ND= No death within the group

Figure 1. Survival of BALB/c Mice at Different Exposure Concentrations of *Y. Pestis*

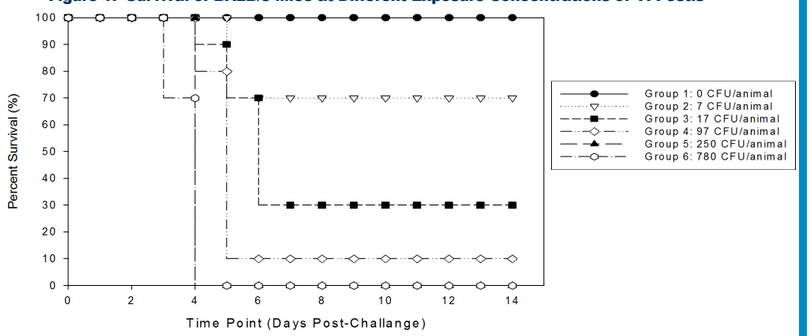


Table 3. Summary of Ciprofloxacin and Doxycycline Results

Model Type	Group	Sample	Inhaled Dose (CFU/animal)	MTD (Days)	Survival (%)
Post-exposure Prophylaxis Model	1	Doxycycline (40 mg/kg)	$1.78x10^5$	2.4	80
	2	Ciprofloxacin (50 mg/kg)		ND	100
	5	None (saline)		3.0	10
Delayed Treatment Model	3	Doxycycline (40 mg/kg)	$1.78x10^5$	2.0	90
	4	Ciprofloxacin (50 mg/kg)		10.7	70
	5	None (saline)		3.0	10

ND= No death within the group

Figure 2. Post-exposure Prophylaxis- Survival of BALB/c Mice After Dosing 24 Hours Post-challenge

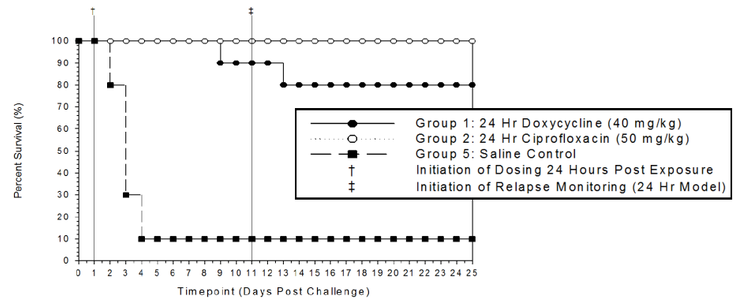
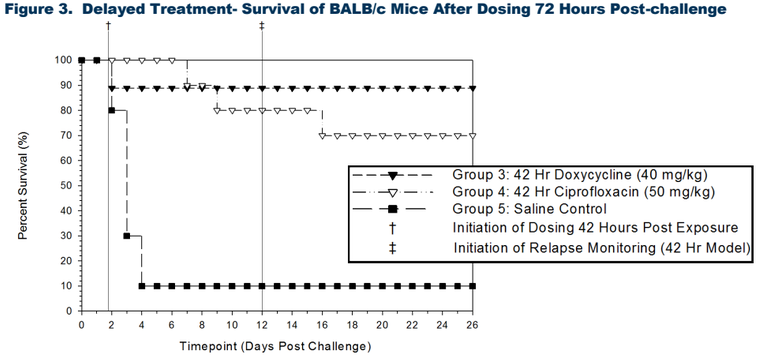


Figure 3. Delayed Treatment- Survival of BALB/c Mice After Dosing 72 Hours Post-challenge



CONCLUSIONS

- LD_{50} was determined to be 8060 CFU/mouse (BALB/c).
- Post-exposure prophylaxis
  - When challenged with *Y. pestis*, all BALB/c mice survived with 50 mg/kg ciprofloxacin when administered within 24 hours post-exposure and dosed four times a day for approximately 10 days. No relapse was observed during the 14 day post-dose period.
  - When challenged with *Y. pestis*, there was 90% survival with 40 mg/kg doxycycline administered at 24 hours post-exposure and dosed four times a day for 10 days. During the relapse period, 60% of the animals survived and 4 additional days, thereafter.
  - No survival was observed in mice that did not receive antibiotic
- Delayed treatment
  - When challenged with *Y. pestis*, approximately 90% of the BALB/c mice survived with 50 mg/kg ciprofloxacin when administered at approximately 42 hours post-exposure and dosed four a day for approximately 10 days. Relapse (70% survival) was observed during the post-dose period.
  - •When challenged with *Y. pestis*, there was 90% survival with 40 mg/kg doxycycline administered at approximately 42 hours post-exposure and dosed four a day for 10 days. During the relapse period, 60% of the animals survived and 4 additional days, thereafter.
  - No survival was observed in mice that did not receive antibiotic.
- Antibiotic treatment yielded significant increase in survival in both the post-exposure prophylaxis and the delayed treatment models. This study demonstrates that treatment with both doxycycline and ciprofloxacin for 10 days is effective in preventing significant relapse of the infections.

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