INFLUENZA A EXPOSURE EXACERBATES PNEUMONIC PLAGUE INFECTION IN SWISS WEBSTER MICE

David A. Boltz, Winston C. Lin, Bruce A. Gingras, Robert O. Baker
IIT Research Institute, Chicago, IL, USA

ABSTRACT

Influenza A viruses are a major cause of human respiratory infections and are responsible for recurrent, seasonal epidemics and pandemics. Research has shown that during past pandemics, secondary bacterial infections contributed to significant morbidity and mortality. Although secondary bacterial infections caused *by S. pneumoniae*, *H. influenza* and *S. aureus* can lead to serious complications, a coinfection with highly virulent bacteria such as the select agent bacterium *Yersinia pestis* following influenza exposure could be catastrophic.

Y. pestis, a Gram-negative bacillus responsible for bubonic plague and pneumonic plague is an important and dangerous bacterial pathogen found in nature that is responsible for sporadic infections throughout the globe. Of greater concern, is the use of *Y. pestis* as a biological weapon. The potential exists that in the event of an intentional release of *Y. pestis*, exposed individuals may have underlying pulmonary diseases or an acute respiratory viral infection, increasing the severity and mortality of pneumonic plague and potentially reducing the efficacy of antibiotic treatment. Therefore, developing a secondary pneumonic plague model is necessary to characterize the pathogenesis of aerosolized *Y. pestis* following viral infection. Our objective was to investigate effects of secondary pneumonic plague on mice infected with influenza A using an efficient nose-only bioaerosol system.

In this study, male and female Swiss Webster mice were inoculated sequentially first with the 0.1 LD_{50} A/Puerto Rico/8/1934 (H1N1) influenza A virus (intranasally) then five days later with *Y. pestis* CO92 via aerosol exposure. Mortality was observed 24-48 hours earlier when *Y. pestis* was preceded by influenza exposure as compared with solely *Y. pestis* infection. The LD_{50} inhaled dose was determined to be $3.5x10^3$ CFU in mice receiving *Y. pestis* alone whereas *Y. pestis* preceded by influenza reduced the LD_{50} of aerosolized *pestis* to 60 CFU. Influenza infection preceding exposure to *Y. pestis* significantly increased the lethality of aerosolized *Y. pestis* in Swiss Webster mice, demonstrating a new model of lethal synergism.

INTRODUCTION

Influenza infections alone are responsible for significant morbidity and mortality; however, research has shown that secondary bacterial pneumonia contributes significantly to morbidity and mortality during influenza epidemics and pandemics. During both the 1918 and 1957 pandemics, the severity of illness and case fatality rate from pneumonia was higher when influenza was followed with bacterial pathogen, compared to the two infections separated. This increased susceptibility to bacterial infections is attributed to the influenza virus predisposing individuals to subsequent bacterial pneumonia and increasing the incidence and severity of secondary bacterial complications. Although secondary bacterial infections with common bacterial strains have been characterized, the pathogenesis of NIAID category A priority bacterial pathogens such as *Y. pestis* post-influenza exposure have yet to be investigated.

Yersinia pestis, a Gram-negative bacteria, is the causative agent for bubonic and pneumonic plague, a rapidly progressing and exceptionally virulent disease. Although co-infections with *Y. pestis* have not been investigated, research on secondary bacterial pneumonia suggests that secondary pneumonic plague could be more severe with increased mortality. And, pre-exposure to influenza (or possibly other respiratory pathogens) could predispose individuals to pneumonic plague. Therefore, developing a secondary pneumonic plague model is necessary to characterize the pathogenesis of aerosolized *Y. pestis* post influenza exposure.

Additionally, this model can be utilized to evaluate treatment options for secondary pneumonic plague; control of primary pneumonic plague is successfully achieved through antibiotics if given early Our ultimate objective is to develop and characterize the secondary pneumonic plague mouse animal model to evaluate the potential lethal synergism between influenza and *Y. pestis*. This development will lead to basic and applied research aimed at the development of vaccines and therapeutics against a potential bioterrorism agent.

METHODS

Male and female Swiss Webster mice were inoculated intranasally with 0.1 LD_{50} of the A/Puerto Rico/8/1934 (H1N1) influenza A virus. Five days post inoculation mice were administered *Y. pestis* CO92 via aerosol exposure. Animals were challenged 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) running concurrently during the exposure at 20 PSI. *Y. pestis* CO92 was grown at 28C to late log-phase (~$2.5x10^8$ CFU/mL).

FIGURE 1. MODIFICATION OF THE PARI LC PLUS® NEBULIZER FOR USE IN THE IITRI BIOAEROSOL SYSTEM

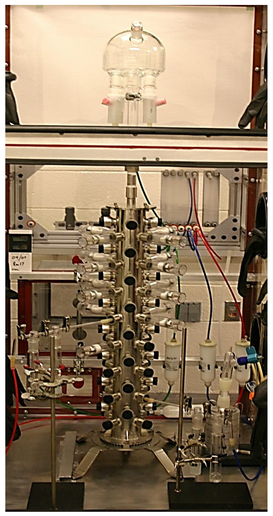
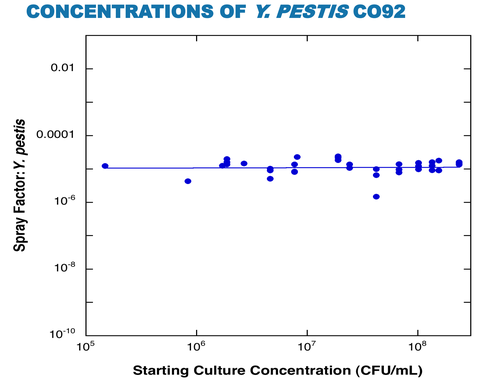


TABLE 1. SUMMARY OF AEROSOL EXPOSURE PERFORMANCE DATA FOR *Y. PESTIS* CO92 USING THE PARI LC PLUS

***Y. pestis* CO92 Concentration**	Target Starting Concentration (CFU/mL)	$1.68x10^5$	$6.77x10^5$	$1.68x10^6$	$3.34x10^6$	$6.75x10^6$
	Initial Titer (CFU/mL)	$1.5x10^5$	$8.4x10^5$	$1.72x10^6$	$2.74x10^6$	$8.2x10^6$
	Post-Challenge Titer (CFU/mL)	$3.4x10^4$	$2.62x10^5$	$2.16x10^6$	$7x10^6$	$1.24x10^7$
	All-Glass Impinger Titer (CFU/mL)	NA	$2.2x10^3$	$1.14x10^4$	$2.34x10^4$	$8.2x10^4$
	Presented Dose (CFU/animal)	$4.8x10^2$	$9.5x10^2$	$5.6x10^3$	$1.0x10^4$	$4.9x10^4$
Particle Size	Particle Diameter (µm)	2.57	1.6	1.56	1.34	1.51
	Geometric SD	1.55	1.22	1.32	1.31	1.27
Spray Factor		$1.16x10^{-5}$	$5.7x10^{-6}$	$1.44x10^{-5}$	$1.8x10^{-5}$	$2.17x10^{-5}$
Average Spray Factor		1.4×10^{-5}				

Note: Run time was 10 minutes for all trials.

FIGURE 2. COMPARISON OF SPRAY FACTORS OVER SEVERAL CONCENTRATIONS OF *Y. PESTIS* CO92



RESULTS

TABLE 2. LD50 DETERMINATION FOR *Y. PESTIS* CO92 IN SWISS WEBSTER MICE

		Males		Females		Overall	
Group	Actual Challenge (CFU/animal)	MTD (Days)	Survival (%)	MTD (Days)	Survival (%)	MTD (Days)	Survival (%)
1	0.00E+00	ND	100%	ND	100%	ND	100%
2	4.80E+02	ND	100%	ND	100%	ND	100%
3	9.50E+02	ND	100%	3.3	40%	3.3	70%
4	5.60E+03	5.0	40%	5.3	20%	5.1	30%
5	1.00E+04	2.2	0%	2.4	0%	2.3	0%
6	4.90E+04	2.4	0%	2.4	0%	2.4	0%

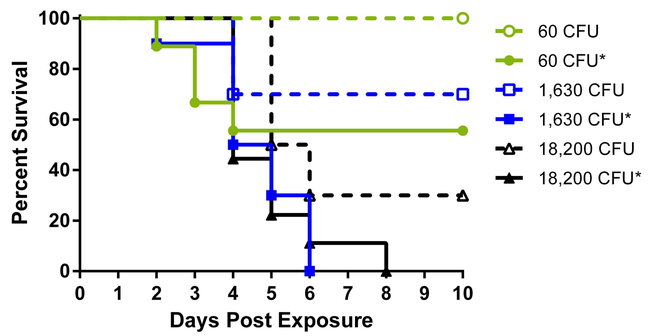
The calculated LD_{50} (Reed-Munch) was determined to be $2.31x10^3$ CFU/animal
NA = Not Determined (due to no death)

TABLE 3. LD50 DETERMINATION FOR *Y. PESTIS* CO92 AFTER EXPOSURE TO INFLUENZA IN SWISS WEBSTER MICE

		Y. pestis		Influenza/*Y. pestis*	
Group	Actual Challenge (CFU/animal)	MTD (Days)	Survival (%)	MTD (Days)	Survival (%)
1	6.00E+01	ND	100%	2.8	55%
2	1.63E+03	3.0	70%	3.7	0%
3	1.82E+04	3.3	30%	3.6	0%

The calculated LD_{50} (Reed-Munch) was determined to be $3.5 x10^3$ CFU/animal for *Y. pestis* alone and 60 CFU/animal for influenza/*Y. Pestis* mice. ND = Not Determined (due to no death)

FIGURE 2. SURVIVAL CURVE FOR TO *Y. PESTIS* +/- INFLUENZA PRE-EXPOSURE IN SWISS WEBSTER MICE



*Significant difference ($p<0.05$) between survival curves within exposure dose. *Y. Pestis* alone (dashed lines) or *Y. Pestis* preceded by influenza virus (sold lines). Control mice receiving only influenza with no exposure to *Y. Pestis* had 100% survival (data not shown).

SUMMARY AND CONCLUSIONS

- LD_{50} for *Y. pestis*-exposed mice was found to be 3.5×10^3 CFU/mouse compared to a LD_{50} for influenza/*Y. pestis*-exposed mice of 60 CFU/mouse.
- Survival rates for Swiss Webster mice exposed to 60 CFU of *Y. pestis* decreased from 100% in the absence of influenza, to 55% for mice previously exposed to influenza.
- 70% of Swiss Webster mice survived with exposure to 1630 CFU of *Y. pestis* in the absence of influenza; however for this same dose 0% survived if the mice were previously exposed to influenza.
- Mortality occurred earlier in influenza/*Y. pestis*-exposed mice compared to mice exposed to *Y. pestis* only.
- These data show that potential synergism between influenza and *Y. pestis*; what remains unclear is how these two pathogens work synergistically.