

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

**020634Orig1s061, 020635Orig1s067,
021721Orig1s028**

MEDICAL REVIEW(S)

Clinical Review Memorandum

NDA: 20-634; 20-635; 21-721
Product: Levofloxacin HCl (Levaquin® IV, tablets, oral suspension)
Sponsor: Janssen Pharmaceuticals (Johnson & Johnson)
Address: 920 US Highway 202, P.O. Box 300
Raritan, NJ 08869-0602
Submission Date: October 27, 2011
Review Date: April 20, 2012
Clarification Date: November 19, 2012
Clinical Reviewer: Yuliya Yasinskaya, MD

The following clarification is provided for information on page 39 of the clinical review (reference ID 3120167, dated April 20, 2012) for sNDAs 20-634, 20-635, and 21-721 for Levofloxacin HCl submitted on October 27, 2011.

Original statement: *DSI inspections conducted on April 24-29, 2011 revealed that the study was of poor quality and lacked integrity, and the inspectors concluded that the study results were not reliable.*

Clarification: *DSI inspections conducted on April 24-29, 2011 documented deviations from the protocol and “confirmed that this was not a GLP study as originally designed and actually conducted”. Therefore, “data quality and integrity are not assured”. The OSI reviewer suggested that “the review division’s discretion be considered for the acceptability of the data for review”. Therefore, the reviewer will present summary data from the study for strictly documentation purposes. The findings from this study might only be included in the final cross natural study analysis and comparison to document consistency or inconsistencies in AGM plague model performance.*

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

YULIYA I YASINSKAYA
11/19/2012

JOHN J ALEXANDER
11/19/2012

CLINICAL REVIEW

Application Number: sNDA 20-634; 20-635; 21-721

Product: Levofloxacin HCl (Levaquin® IV, tablets, oral suspension)

Sponsor: Janssen Pharmaceuticals (Johnson & Johnson)

Address: 920 US Highway 202, P.O. Box 300
Raritan, NJ 08869-0602

Submission Category/Title: SNDA

Submission Receipt Date: October 27, 2011

Review Completion Date: January 31, 2012

Clinical Reviewer: Yuliya Yasinskaya, MD

Clinical Team Leader: John Alexander, MD MPH

Submission contents reviewed:

Study report and accompanying study data “Natural Course of Untreated Pneumonic Plague in African Green Monkeys”, Battelle Study Protocol 617-G607610

Study report and accompanying study data “Natural Course of Untreated Pneumonic Plague in African Green Monkeys”, Battelle Study 875-G607610 with audited animal data

Study report and accompanying study data “A Natural History Study of Inhalational Plague *Y. pestis* Strain CO92 in Adult Telemetered African Green Monkey”, Lovelace Respiratory Research Institute (LRRI) Study Protocol FY06-126 (NIAID protocol D13-1)

Study report and accompanying study data “A Natural History Study of Pneumonic Plague in African Green Monkey”, U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) Study Protocol FY03-09G

Background:

The submission under review contains 4 study reports of the natural history studies of pneumonic plague in African Green Monkeys in support of the NDA efficacy study for levofloxacin for pneumonic plague indication under the regulatory provisions of the Animal rule, 21 CFR 314.600-650 (subpart I).

Final study report “Natural Course of Untreated Pneumonic Plague in African Green Monkeys”, Battelle Study Protocol 617-G607610

Study Facilities

This study was performed at the Battelle Biomedical Research Center (BBRC), Jefferson, OH. Animal quarantine and a 20-day recovery period post surgical implantation of telemetry units took place at the Columbus, OH Battelle facility. Histopathology preparations and evaluations were also performed at the Columbus Battelle facilities.

Y. pestis challenges of African Green monkeys (AGM; *Chlorocebus sabaues*) were conducted in a Biosafety Level 3 (BSL-3) containment laboratory at the BBRC registered with the Centers for Disease Control and Prevention Select Agent program.

Study Animals

Fourteen symptom and malformation-free AGM (4 males, 10 females) were obtained from NIH Yamasse, SC animal facility. Animals were screened for previous exposure to environmental *Yersinia* (i.e., *Y. pseudotuberculosis* and *Y. enterocolitica*) with a non-validated, semi-quantitative memory B-cell assay for cells reacting to *Yersinia* F1 and V antigens. 5/14 of the tested animals were found to be weakly positive for F1 antigen specific B cells and 7/14 were weakly positive for V-antigen specific B cells. 11/14 animals were positive for rotavirus SA11 antibodies. All animals underwent surgical implantation of telemetry devices and were in receipt of prophylactic enrofloxacin antibiotic for 7 days post implantation. One animal did not survive the implantation. One animal (X776) was excluded from the selection process due to transient health issues. Ten study animals (all 3 surviving males and 7 random females) were randomized to the challenge order, while other three other animals were held in reserve. Animals were identified by a tattoo and individual cage cards.

Challenge Agent

Yersinia pestis strain CO92 was the challenge agent in this study. Organism was administered via nebulized aerosol in the head-only exposure chamber with a flow rate of ~16L/min. All 10 animals were anesthetized and challenged on July 17, 2007. The exposure was targeted at x100 LD₅₀ (340 cfu of *Y. pestis*)¹. Please refer to Clinical Microbiology review by Dr. Simone Shurland and Pharmacology Toxicology review by Dr. Stephen Hundley for the detailed assessment of the stock pathogen, aerosol procedures, and calculation of the challenge doses for individual animals.

Study Schedule

The animals were observed twice daily from Day -7 pre-challenge until death according to Battelle SOP MREF.VII-010. Body temperature, ECG activity, and cardiovascular function were monitored at least 30 seconds every hour for 7 days pre-challenge (baseline) and at a minimum of 30 seconds every hour during the post-challenge observation period. Animals judged to be

¹ Adamovicz, J. J, and Worsham, P.L. (2006). Plague in *Biodefense research methodology and animal models*, J. R. Swearingen, Ed. (CRC Press, Boca Raton, FL, 2006) ,chapter 8, pp. 107-135.

moribund were euthanized. For animals found dead the time of deaths was estimated within ± 15 min based on the recorded telemetry data (HR, RR, BP systolic, BP diastolic, temperature, and pulse pressure). Weights were recorded on Day -3 and at necropsy. Complete gross necropsies were performed on any animal found dead or euthanized to confirm death or illness due to *Y. pestis* infection. Fluid, from any AGM observed to have frank, copious secretions in its upper or lower respiratory tracts, was collected on sterile swabs and cultured on CIN (*Yersinia* selective) agar. Brain, heart, kidneys, liver, spleen, lungs, intrathoracic lymph nodes (bronchial and mediastinal), and gross lesions were collected in 10% neutral buffered formalin for histopathology by a board-certified veterinary pathologist. Blood samples were collected daily for chemistry and hematology starting on D 0 post challenge while blood collections for bacteremia started on Day 1 post challenge. Additional sample was collected at the time of terminal bleed.

Table 1 Study Schedule

N of AGM	Study Day						
	Aerosol Challenge	Telemetry	Hematology	Clinical chemistry	Bacteriology	ELISA	Necropsy
10	0	0-21	0 _§ , 1, 2, 3, 4	0 _§ , 1, 2, 3, 4	1, 2, 3, 4, 21*	0 _§ , 21*	21

§ Prior to challenge

* Planned but not needed since all animals died prior to Day 21

Adapted from the study report

MO comment: Unfortunately, the study protocol collected bacteremia samples only once a day (time not specified) precluding any analyses for derivation of therapeutic window and or trigger for intervention. In addition the draft study report does not contain telemetry listings for individual animals. The telemetry graphs provided in the draft report are difficult to analyze, i.e. to determine temperature change over baseline precisely, as the time course is presented in days rather than hours and temperature units on Y axis are compressed to every two degrees for some animals. The time of challenge for each animal is also not provided in the report; therefore, time to death assessments are also done imprecisely (cut of for time of challenge on the graph was used as 12 noon for all animals). The report review, therefore, will primarily focus on the review of the summary clinical observation and laboratory data.

Study Results

Animals were exposed to *Y. pestis* bacterial aerosol via head only inhalation. Inhaled dose cfu and LD50 were calculated based on the concentration of the nebulizing stock, tidal volume and respiratory rate of an animal, air flow rate, concentration of the bacteria in the impinger, sampling volumes, and exposure duration.

Table 2 Aerosol Exposure

Animal ID	Sex	Weight (kg)	Inhaled Dose (cfu)	Inhaled Dose (LD50)
X106	M	4.02	3.75×10^5	1102
X396	M	4.69	3.93×10^5	1154
X421	F	3.74	1.94×10^5	572
X434	F	4.16	2.86×10^5	841
X511	F	3.88	0.798×10^5	2353
X515	F	3.7	3.51×10^5	1032
X606	M	4.20	0.36×10^5	106
X711	F	3.67	1.76×10^5	518

X759	F	3.36	0.98×10^5	288
X770	F	3.69	1.09×10^5	324
Mean±SD		3.9±0.4	$2.09 \times 10^5 \pm 1.32 \times 10^5$	614±389
Median		3.8	1.85×10^5	545

Reviewer noted minor differences (0-3000 cfu and 2-8 xLD50) between calculated inhaled doses in the dataset AEROSOL and the study report. The table reflects calculated aerosol data as presented in SAS datasets submitted along the study report.

MO comment: *The exposure doses in this experiment exceeded target x100 LD50 dose by several orders of magnitude. It is not clear whether such excessive exposure had an impact on the duration of illness in the animals. The only animal that had target exposure achieved appeared to survive the longest. The methodology of the exposure might need to be revised to achieve more consistent and targeted delivery of the dose.*

Table 3 Clinical Observations Post Challenge

	ID	X106	X396	X421	X434	X511	X515	X606	X711	X759	X770
Date	Sex	M	M	F	F	F	F	M	F	F	F
7/17/2007	PM	N	N	NE	N	N	N	N	N	NE	NE
7/18/2007	AM	N	NE	NE	V, NE	N	N	N	N	NE	NE
7/18/2007	PM	N	NE	NE	N	N	NE	N	N	NE	NE
7/19/2007	AM	NE	BND BDM, FD 00:00	NE	NE	NE	NE	N	NE	NE	NE
7/19/2007	PM	NE, NS		NE	NE	NE	NE	NE	NE	NE, NS	NE, NS
7/20/2007	AM	RD, NE,OB		BND, BDM, FD 7:00	BND, BDM, FD 5:00	FD 6:00	BND, BDM, FD 7:00	NE	BND, BDM, FD 8:53	NE	BND, FD 8:00
7/20/2007	PM	RD, NE, OB						NE		NE	
7/21/2007	AM	FD 01:00						FD 6:40		L,P,H, NE, RD, M, FD 7:41	

Animals challenged with *Y. pestis* the morning of 7/17/07 after clinical observations

BDM = Bloody discharge from mouth

N = Normal

BND = Bloody nasal discharge

NE = Not eating

FD = Found dead

Modified from the study report

NS = No stool

H = Hunched posture

OB = Open mouth breathing

L = Lethargic

RD = Respiratory distress

M = Moribund

V = vomited

P = prostrate

MO comment: *The symptoms that manifested itself at the time of temperature elevation were prolonged (≥ 24 h) loss of appetite. Other symptoms like nasal and respiratory secretions, oral breathing, lethargy, respiratory distress, and hunched posture were noted immediately prior to death. Again, as positive infection, i.e. bacteremia, lung infiltrates, were assessed infrequently or not at all, the correlation between loss of appetite and disease onset, documented by positive blood culture, as a trigger for intervention cannot be established.*

Telemetry

A significant increase in the heart rate was observed in all animals beginning at approximately 2 days post-challenge. This was accompanied by a slight but sustained increase in blood pressure: both systolic and diastolic, and pulse pressure. Body temperature demonstrated diurnal variation

from 36 to up to 39 °C prior to challenge and then increased to a sustained temperature of > 40 °C approximately 1.5-2 day post challenge.

Table 4 Hourly Average Baseline Temperature, C°

Hour of the Day	X106	X396	X421	X434	X511	X515	X606	X711	X759	X770
0	37.11	36.88	36.59	36.76	36.56	36.74	36.97	36.89	36.69	36.63
1	37.02	36.89	36.60	36.77	36.60	36.77	36.97	36.93	36.80	36.65
2	36.85	36.83	36.66	36.88	36.72	36.57	37.00	37.01	36.84	36.66
3	36.93	36.91	36.79	36.85	36.77	36.65	37.04	37.03	36.85	36.72
4	37.16	36.72	36.56	37.08	36.74	36.73	37.15	37.08	36.87	36.76
5	37.44	36.56	36.73	37.22	36.93	36.92	37.39	37.25	37.12	36.91
6	37.86	37.06	37.01	37.49	37.29	37.52	37.81	37.58	37.56	37.08
7	38.31	37.77	37.51	37.93	37.76	37.93	38.20	38.02	38.12	37.65
8	38.49	38.20	38.09	38.14	37.74	38.14	38.44	38.37	38.56	38.12
9	38.46	38.09	37.87	38.19	37.53	37.80	38.38	38.15	38.42	37.93
10	38.55	38.06	38.00	38.16	37.67	37.95	38.40	38.15	38.30	37.82
11	38.48	38.16	38.10	38.13	37.89	37.75	38.36	38.08	38.27	37.77
12	38.43	38.12	37.85	38.21	37.77	37.65	38.25	37.90	38.40	37.88
13	38.47	38.17	37.89	38.14	37.87	37.84	38.44	37.96	38.06	37.92
14	38.63	38.24	38.12	38.16	37.82	37.96	38.32	38.09	38.36	37.92
15	38.65	38.14	38.15	38.06	37.90	37.98	38.46	38.22	38.35	38.02
16	38.45	38.11	38.18	37.73	37.85	37.85	38.30	38.12	38.31	38.02
17	37.60	37.41	37.68	37.11	37.63	37.35	37.63	37.91	38.02	37.82
18	37.07	37.03	37.10	36.88	37.28	36.84	37.28	37.12	37.47	37.44
19	37.31	37.04	36.72	36.72	36.91	36.67	37.25	36.79	36.91	37.28
20	37.20	37.05	36.82	36.58	36.79	36.78	37.15	36.81	36.73	37.09
21	36.90	37.00	36.64	36.74	36.69	36.74	37.12	36.83	36.67	36.94
22	37.06	36.93	36.53	36.70	36.67	36.65	37.01	36.86	36.64	36.78
23	36.88	36.90	36.50	36.67	36.57	36.65	37.01	36.84	36.60	36.66

In this study protocol **fever was defined as the first of three consecutive hourly measurements at least 1.5C above their respective baseline averages at the same hour of the day.** For most animals temperature elevations have occurred between 36-48 hours following *Y. pestis* challenge. The temperature remained elevated until just prior to death.

Table 5 Fever Onset

Animal ID	Time from Challenge to Onset of Fever (hh:mm)	Onset of Fever Date and Time	Challenge Date and Time	Temperature at Onset of Fever (C)	Baseline Average Temperature (C)	Baseline Average Temperature + 1.5 (C)
X106	38:49	19JUL07 02:00	17JUL2007 11:10:55	38.78	36.85	38.35
X396	41:12	19JUL07 04:00	17JUL2007 10:48:24	38.33	36.72	38.22
X421	45:22	19JUL07 09:00	17JUL2007 11:38:00	40.06	37.87	39.37
X434	48:12	19JUL07 13:00	17JUL2007 12:48:02	39.80	38.14	39.64
X511	49:29	19JUL07 14:00	17JUL2007 12:30:36	40.09	37.82	39.32
X515*	36:35	18JUL07 23:00	17JUL2007 10:24:32	38.65	36.65	38.15
X606	44:31	19JUL07 10:00	17JUL2007 13:29:12	40.30	38.40	39.90
X711	44:56	19JUL07 09:00	17JUL2007 12:04:23	40.24	38.15	39.65
X759	45:43	19JUL07 11:00	17JUL2007 13:16:55	40.04	38.27	39.77
X770	43:02	19JUL07 09:00	17JUL2007 13:58:02	39.98	37.93	39.43

* For Animal X515, some unavailable telemetry readings were not available. This was the first time a three hours block was over the limit, but the middle hour measurement was missing

Animal ID	Time from Challenge to Onset of Fever (hh:mm)	Onset of Fever Date and Time	Challenge Date and Time	Temperature at Onset of Fever (C)	Baseline Average Temperature (C)	Baseline Average Temperature + 1.5 (C)
X515	44:35	19JUL07:07:00	17JUL2007:10:24:32	40.54	37.93	39.43

* For animal X515, this is the onset under the requirement that three consecutive measurements (not necessarily continuous time) were at least 1 .5C above their respective baseline averages at the same hours of the day

MO comment: *Temperature elevations in all animals to 40C or sustained (>2h) elevations >1.5C over baseline, appear to be the sign of the disease corresponding to fever in humans. The definition of fever employed is acceptable as significant diurnal variations have been observed at baseline in NHP. Fever as a sign of disease was consistently present by 50 hours post challenge in all animals and remained present until animal death (see Figure 1). As the temperature remained elevated until time of death the time from this sign onset to the time of animal death can, therefore, serve as a window for therapeutic intervention. However the major drawback of the study is that blood cultures were collected only once daily; therefore, the correlation between the temperature elevation and the onset of bacteremia (microbiological surrogate of the disease) could not be established.*

Little change was observed in the QT interval or activity post-challenge. From approximately 2 days post-challenge to death a marked increase in respiratory rate was accompanied by a decrease in inspiratory and expiratory time, indicative of shallow rapid breathing. The RP integral, a measure of tidal volume, tended to increase slightly and showed less variation starting approximately 1.5 to 2 days post-challenge and remaining fairly constant until death.

Laboratory findings

Hemoglobin and RBC values decreased as compared to baseline by study Day 2. Increases in hematocrit values were noted on Day 1. Initial rise in platelet count on Day 1 was followed by a decrease on Day 3. WBC parameters exhibited the following pattern: neutrophilic leukocytosis was observed by study Day 2 along with significant increase in neutrophil/lymphocyte ratio. Monocyte, basophil, and eosinophil percentages also increased relative to baseline as disease progressed (Day 2).

Table 6 Hematologic Parameters, Changes from Baseline, Study Day

Hematology Parameter	Study Day 1		Study Day 2		Study Day 3	
	N	Mean Shift	N	Mean Shift	N	Mean Shift
Red Blood Cell Count ‡	10	1.015	10	0.956 ↓	3	0.912
Hemoglobin ‡	10	1.000	10	0.952 ↓	3	0.904
Hematocrit ‡	10	1.047 ↑	10	0.967	3	0.912
MCV	10	2.380 ↑	10	0.830	3	-0.033
MCH ‡	10	0.985 ↓	10	0.995	3	0.990
MCHC	10	-1.450 ↓	10	-0.500	3	-0.333
RDW ‡	10	0.998	10	1.008	3	1.028
Platelet Count	10	36.200 ↑	10	7.400	3	-98.333 ↓
MPV ‡	10	1.010	10	1.023	3	0.977
White Blood Cell Count ‡	10	1.526 ↑	10	2.325 ↑	3	2.235
Neutrophils ‡	10	1.455 ↑	10	4.060 ↑	3	3.152
Lymphocytes ‡	10	1.587 ↑	10	0.792	3	1.469
Neutrophils/Lymphocytes Ratio ‡	10	0.917	10	5.126 ↑	3	2.147 ↑
Monocytes ‡	10	1.893 ↑	10	2.130 ↑	3	1.931
Eosinophils ‡	10	1.881 ↑	10	1.014	3	0.920
Basophils ‡	10	2.169 ↑	10	2.685 ↑	3	5.604

‡ Distribution was log-normal for this parameter.

↑, ↓ Indicates that, for untransformed data, the difference between baseline and study day means was significantly different from zero (at the 0.05 level); for log-transformed data, the ratio of geometric means at baseline and study day was significantly different from 1 (at the 0.05 level). "↑" indicates that the mean at the study day was greater than baseline, while "↓" indicates that the mean at the study day was less than baseline.

Adapted from the study report

MO comment: Changes in hematology parameters noted in animals post challenge, specifically neutrophilic leukocytosis, are characteristic of inflammatory/infectious process and correspond to the changes observed in humans.

Significant clinical chemistry changes were noted in challenged animal as well: AST, LDH and creatinine had all increased on Day 1 and continued their upward trend until animal succumbed to death. Reverse was true for chloride.

Table 7 Clinical Chemistry Parameters, Changes from Baseline, Study Day

Clinical Chemistry Parameter	Study Day 1		Study Day 2		Study Day 3	
	N	Mean Shift	N	Mean Shift	N	Mean Shift
Total Bilirubin	10	-0.020	10	-0.020	5	0.040
Aspartate Aminotransferase ‡	10	1.321 ↑	10	1.360 ↑	5	11.487 ↑
Alanine Aminotransferase ‡	10	1.224 ↑	10	1.146	5	3.738
Lactate Dehydrogenase ‡	10	0.984	10	1.635 ↑	4	5.184 ↑
Total Protein ‡	10	1.031 ↑	10	0.984	5	0.945
Blood Urea Nitrogen ‡	10	1.163 ↑	10	0.831 ↓	5	1.302
Creatinine ‡	10	1.511 ↑	10	1.418 ↑	5	2.276 ↑
Sodium	10	3.500 ↑	10	1.600	6	-14.333
Potassium ‡	10	1.134 ↑	10	0.994	6	2.690
Chloride	10	-3.700 ↓	10	-2.200	6	-16.000 ↓
Calcium	10	0.946 ↑	10	-0.003	5	-0.188
Phosphorus ‡	10	1.264 ↑	10	1.087	5	1.909

‡ Distribution was log-normal for this parameter.

↑, ↓ Indicates that, for untransformed data, the difference between baseline and study day means was significantly different from zero (at the 0.05 level); for log-transformed data, the ratio of geometric means at baseline and study day was significantly different from 1 (at the 0.05 level). "↑" indicates that the mean at the study day was greater than baseline, while "↓" indicates that the mean at the study day was less than baseline.

Adapted from the study report

MO comment: Abnormalities in liver and kidney function tests are common occurrence in human plague. The findings in animals generally are similar to those seen in humans. Unfortunately, again, blood samples for CBC and chemistry were collected daily and the correlation between the changes in laboratory parameters and the onset of bacteremia and fever could not be established.

Bacteremia

Y. pestis bacteremia was detectable in all surviving animals on Days 2 and 3 post-challenge. All animals were dead by Day 4 post-challenge. CRA plates were visually inspected for colony morphology consistent with *Y. pestis*. On day 1 post-challenge, none of the animals demonstrated detectable *Y. pestis* in blood. Culture of blood from most of the animals on Days 2 and 3 post-challenge and at the terminal bleed had pigmented colonies characteristic of *Y. pestis*. Contamination with other organisms was also noted for 7 of 10 terminal bleeds.

Table 8 Quantitative Bacteremia

AGM #	F1/V positivity pre-challenge	Challenge dose	Day 1	Day 2	Day 3	Terminal
X106	-/+	3.75 × 10 ⁵	0.00	3.06E+04	7.70E+06	Positive
X396	-/+	3.93 × 10 ⁵	0.00	6.57E+05		Positive, contaminant present
X421	-/-	1.94 × 10 ⁵	0.00	3.76E+03		Positive, contaminant present

AGM #	F1/V positivity pre-challenge	Challenge dose	Day 1	Day 2	Day 3	Terminal
X434	-/-	2.86×10^5	0.00	8.47E+03		Positive, contaminant present
X511	+/+	0.798×10^5	0.00	3.97E+04		Positive, contaminant present
X515	+/-	3.51×10^5	0.00	5.97E+04		Positive, contaminant present
X606	-/-	0.36×10^5	0.00	1.26E+04	2.54E+04	Positive
X711	+/-	1.76×10^5	0.00	4.37E+04		Negative, contaminant present
X759	-/-	0.98×10^5	0.00	3.30E+01	2.14E+07	Positive
X770	+/-	1.09×10^5	0.00	6.23E+04		Positive, contaminant present

Modified from the study report

MO comment: For animals where sequential cultures were obtained, there was log increase in cfu of *Y. pestis*. Contamination of the terminal blood cultures is unfortunate and could be explained by internalization of the gut bacteria as animals remained at room temperature for prolonged period of time (up to 12 hours) after death. There does not appear to be a correlation between the aerosol exposure dose and the onset of bacteremia in days or the degree of bacteremia on Day 2 or bacteremia duration prior to death. Previous weak positivity of memory B-cell assay for F1 and V antigens in some animals did not have an effect on the onset, degree, or duration of bacteremia or the outcome of death in this study. Animal X711 had negative terminal blood culture, but *Y. pestis* was identified in the lung, mediastinal and spleen tissue on histopathology.

As presented during NIAID plague animal model annual update on 02/24/2011 onset of bacteremia prior to onset of fever was documented in 2/10 animals. Average time to first positive blood culture was 46 hours (range 44-47h).

Other microbiology results

If an animal had respiratory secretions anytime post exposure they were collected and processed for culture.

Table 9 Nasopharyngeal and Lung washings cultures

AGM #	Date of Death	Nasopharyngeal Discharge	Lung Fluid
X106	7/21/2007	+	+
X396	7/20/2007	+	+
X421	7/20/2007	+	+
X434	7/20/2007	+, contaminant	+
X511	7/20/2007	+	+
X515	7/20/2007	+	+
X606	7/21/2007	No sample	No sample
X711	7/20/2007	+, contaminant	Weak +, contaminant
X759	7/21/2007	No sample	+
X770	7/20/2007	+	+

Contaminants were not identified

Adapted from the study report

MO comment: The contamination of the nasopharyngeal and lung washings material is not unexpected. All, but one animal had respiratory secretions. The respiratory secretions collected were *Y. pestis* positive.

Timing of death (TOD)

Respiratory rate recordings were used as the primary criteria for TOD determinations based on telemetry data. When respiratory rate values decreased and were no longer recorded, a “No Data” message was produced, which was a good indicator of the TOD. If respiratory data were not available or did not clearly indicate a TOD, temperature was used as the secondary parameter. TOD obtained from temperature was determined when the temperature was < 36°C. Blood pressure recordings < 5 mmHg were also used to support TOD determinations. Telemetry blood pressure values are calculations equal to the average of systolic and diastolic blood pressures for each 30 sec time interval.

In this study animals survived to 61-90 h post challenge.

Table 10 Time to fever and death (calculated from telemetry dataset)

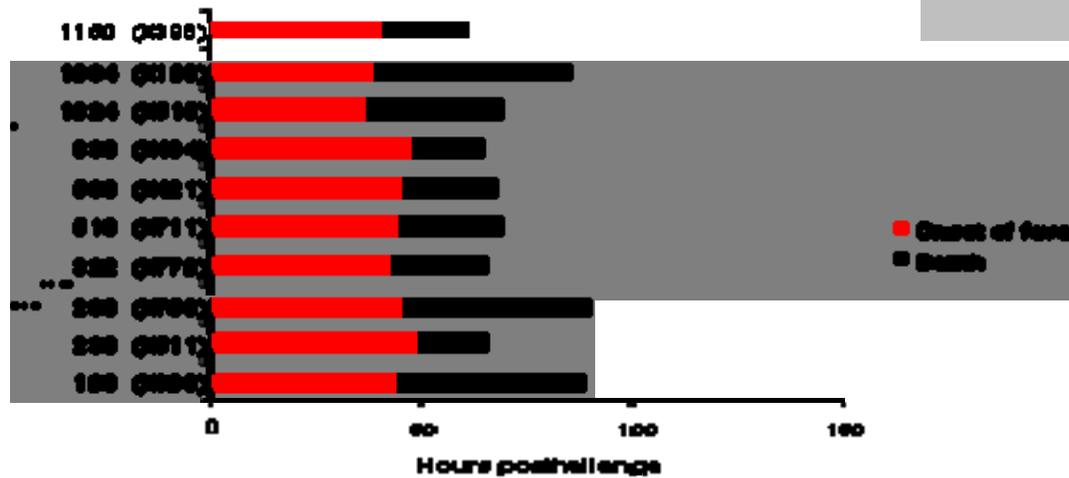
AGM #	Challenge dose	Time to onset of fever (h)	Time from fever onset to death (h)	Time to death (h)
X106	3.75×10^5	39	44	83
X396	3.93×10^5	41	20	61
X421	1.94×10^5	45	22	67
X434	2.86×10^5	48	16	64
X511	0.798×10^5	49.5	16	65.5
X515	3.51×10^5	36.5	32	68.5
X606	0.36×10^5	44.5	44.5	89
X711	1.76×10^5	45	24	69
X759	0.98×10^5	45.5	44.5	90
X770	1.09×10^5	43	23	66
Mean±SD	$2.09 \times 10^5 \pm 1.32 \times 10^5$	43.7±3.9	28.6±11.7	72.3±10.7
Median	1.85×10^5	44.8	23.5	67.8

MO comment: In this study the onset of fever for most of the animals happened between 36 and 48 hours post challenge (43.7 hours average). From the time of fever onset and death animals lived for a short period of time: with 50% of the animals dying within 24 hours after onset of fever (average 28.69 hours). Therefore, it appears that therapeutic intervention in this animal model needs to be evaluated fairly close to the onset of fever. From the report all the animals were bacteremic on day 2 of the study. Time of the blood culture collection was not provided in the report. The reviewer is unable to estimate how long animals survived after they were determined to be bacteremic. The correlation between the time to death and onset of bacteremia or any other laboratory parameter changes cannot be established due to infrequent blood sampling. Although animals X606 and X759 (received 2 of the lowest challenge doses) survived the longest, the third longest survival was observed in animal X106 that received the second highest dose on the study. There was no correlation between the challenge dose and the onset of fever and/or death.

A graphical presentation of onset of fever, defined as temperature $\geq 1.5^\circ\text{C}$ above baseline and sustained for >2 hours, and death was provided by Battelle during NIAID plague animal model update on 02/24/2011.

Figure 1 Onset of fever and death (hours post challenge)
Battelle Study 6-17

Best Available Copy



Adapted from the Battelle presentation at the annual NIAID plague animal model update held on 02/24/2011

MO comment: Onset of fever and time to death did not correlate with the challenge dose (Y axis of the above graph). Average incubation period (time to fever onset) for this study was $43.7 \pm 3.9h$ (range from 36 hours to 49h). Again average time to death from the time of fever onset was approximately $28.6 \pm 11.7h$ (range from 16 to 44 hours). Duration of symptoms prior to death in human pneumonic plague described in the literature does not usually exceed 3 days

Pathology

All monkeys died had lesions consistent with pneumonic plague on necropsy. Common findings in the lung included bacteria both within macrophages and extracellularly, associated with suppurative inflammation and variable amounts of fibrin accumulation, hemorrhages in most animals and occasionally edema. Bacteria, usually associated with hemorrhage and suppurative inflammation, were also noted in almost all of the lymph nodes examined, especially bronchial lymph nodes. Fibrin thrombi in renal glomerular capillaries were noted in only one AGM (male X106) in this study.

MO comment: While time of death could be ascertained from the telemetry tracings, it is disconcerting to note that all 10 animals were found dead and none was euthanized. Clinical observations conducted more often than protocol specified bid intervals might have allowed timely relief pain and suffering of the research animals.

The table below depicts the findings on microscopic examination of organs of all challenged animals.

Table 11 Histopathology Findings

ID	X106	X396	X421	X434	X511	X515	X606	X711	X759	X770
Tissue/Sex	M	M	F	F	F	F	M	F	F	F
Death	Found dead	Found dead	Found dead	Found dead	Found dead	Found dead	Found dead	Found dead	Found dead	Found dead
Heart	unremarkable	Protozoal cyst +	unremarkable	unremarkable	unremarkable	Myocardial fibrosis +	unremarkable	unremarkable	unremarkable	unremarkable
LN bronchial	bacteria +++++	bacteria +++	bacteria +++++ hemorrhage + inflammation ++	bacteria +++ necrosis + hemorrhage +	bacteria +++	bacteria +++ hemorrhage ++ inflammation ++	bacteria ++ inflammation ++	bacteria ++ hemorrhage + inflammation +	bacteria ++ hemorrhage +	bacteria +++++ hemorrhage ++ inflammation +++
LN mediastinal	bacteria +++++ hemorrhage ++	bacteria +++++ hemorrhage +++	bacteria ++ hemorrhage +	bacteria ++ inflammation +	bacteria ++	bacteria ++ hemorrhage +++++	bacteria ++ inflammation ++	bacteria ++	unremarkable	bacteria +++ hemorrhage ++
Lung	intra and extra cellular bacteria +/+ +++++, hemorrhage ++, inflammation +++	intra and extra cellular bacteria +/+ +++++, inflammation ++	intra and extra cellular bacteria +/+ +, hemorrhage +, inflammation ++	intra and extra cellular bacteria +/+ +++++, hemorrhage ++	intra and extra cellular bacteria +/+ +, inflammation ++	intra and extra cellular bacteria +/+ +++++, hemorrhage +, inflammation ++	intra and extra cellular bacteria +/+ +++++, hemorrhage +++++, inflammation +++++, edema+++	intra and extra cellular bacteria +/+ +++++, hemorrhage +, inflammation ++, edema+	intra and extra cellular bacteria +/+ +++++, hemorrhage ++, inflammation +++++	intra and extra cellular bacteria +/+ +++++, inflammation ++
Spleen	unremarkable	bacteria +++, necrosis ++	intra extracellular bacteria +++++/+	bacteria intracellular +++++	bacteria intra extracellular +++++/+ necrosis +	unremarkable	necrosis ++	bacteria + necrosis +	bacteria intracellular +	bacteria intracellular ++
LN mandibular	bacteria +++, hemorrhage+	bacteria +++, hemorrhage++	unremarkable	unremarkable	unremarkable	unremarkable	unremarkable	bacteria +++++ hemorrhage + inflammation ++	unremarkable	unremarkable
Thoracic cavity	red fluid 15 ml	clear fluid 15 ml	clear fluid 7 ml	clear fluid 20 ml	clear fluid 15 ml	clear fluid 3 ml		clear fluid 15 ml	clear fluid 10 ml	clear fluid 10 ml
Other organs	Kidneys: glomerular thrombi +	unremarkable	unremarkable	unremarkable	unremarkable	unremarkable	unremarkable	unremarkable	unremarkable	Kidneys: ++ crystals microgranuloma tubulointerstitial nephritis ++

MO comment: Histopathology findings in all the animals are consistent with pneumonic plague and are similar to those findings described on autopsy of the humans who died of pneumonic plague.

Conclusions:

The study results demonstrate that the inhalational model of pneumonic plague in AGM is uniformly fatal at exposures of >100 LD₅₀ (3.59×10^4 cfu of *Y. pestis* CO92). All animals develop elevated temperature response to the disease approximately 48-50 hours post exposure and then succumb to the disease 24-48 hours later. Fever and loss of appetite are the most consistent signs of the disease in these species. Tachycardia and tachypnea observed in the animals are notable at the time of fever onset. All animals became bacteremic on Day 2 post challenge with increasing titers until death. Nearly all animals developed respiratory secretions (found to be positive for *Y. pestis*) shortly before death. Upon necropsy/histopathology examination changes in lungs, mediastinal lymph nodes were characteristic of pneumonic plague. Pathogen, time course of the disease, clinical presentation, laboratory as well as histopathology findings are sufficiently similar to those described for human pneumonic plague. As in humans window for therapeutic intervention in AGM appears to be short (28-29h average); thus, the treatment is likely to be most beneficial when administered shortly after symptom manifestation, i.e. fever, rather than upon confirmation of blood culture positivity. It also appears possible that the majority of animals will be bacteremic at the fever onset and the collection of blood for culture after fever onset prior to treatment administration will allow selection of animals for a conservative (microbiologically confirmed) efficacy analysis population.

Study report “Natural Course of Untreated Pneumonic Plague in African Green Monkeys”, Battelle Study Protocol 875-G607610

Animals

Twelve specific-pathogen-free symptom and malformation free AGMs (*Chlorocebus aethiops*) were provided by NIAID for this study. Ten (5 male, 5 female) AGMs weighing 3.3-5.3 kg were placed on the study. Two female AGMs were available as replacements prior to the day of challenge. Animals were initially quarantined, then surgically implanted telemetry devices and allowed to recover for 14 days post surgery. All AGMs were screened by ELISA for previous exposure to *Y. pestis* and for environmental Yersiniae that are immunologically similar to *Y. pestis*, i.e. *Y. pseudotuberculosis* and *Y. enterocolitica*. Animals were identified by tattoos and individual cage cards.

Study Design

On study Day 0 animals were challenged with a targeted aerosol dose of 100×LD50 of *Y. pestis* strain CO92 (approximately [redacted] inhaled cfu). The estimated aerosol LD50 in AGMs was reported to be [redacted]. Clinical observations were conducted twice daily for approximately two weeks prior to challenge and three times a day (approximately 8 hours apart) for the first four days following challenge. Observations were continued three times daily after study Day 4 if animals were febrile. The study veterinarian determined times of death from a review of the clinical observations and the telemetry data. Telemetry parameters were utilized to determine time of death within a 5-138 minute window. Body temperature, respiration, ECG activity, and cardiovascular function were monitored at least 30 seconds every 15 minutes for thirteen days pre-challenge (baseline) and at a minimum of 30 seconds every 15 minutes during the post-challenge observation period until animal death.

Table 12 Study Schedule

N of AGM	Study Day							
	Aerosol Challenge	Telemetry ¹	Hematology ⁵	Clinical chemistry ⁵	Coagulation ⁵	Bacteriology	ELISA ²	Necropsy
10	0	0-21	0 ³ , 1, 2, 3, 4, 5	0 ³ , 1, 2, 3, 4, 5	0 ³ , 1, 2, 3, 4, 5	1, 2, 3, 4, 5, 21 ⁵	0 ⁴ , 21 ⁵	21

¹ Baseline (pre-challenge) telemetry was acquired for thirteen days prior to challenge. Post-challenge telemetry and routine clinical observations occurred until the day of death.

² To document the lack of pre-existing antibodies to *Yersinia* F1 or V antigens and to confirm infection in animals challenged with *Y. pestis*, if necessary.

³ Prior to challenge.

⁴ During quarantine and, if possible, from animals that were found dead.

⁵ Blood samples also were obtained from animals that succumbed to the challenge prior to Study Day 21. Necropsies were performed on animals that succumbed to challenge. Additional unscheduled blood samples were obtained from AGMs that showed an elevated temperature after Study Day 4.

Adapted from the study report.

MO comment: Unfortunately, the study protocol collected bacteremia samples only once a day (time not specified) precluding any analyses for derivation of therapeutic window and or trigger for intervention. The results of screening serology for F1 and V antigens were not provided in the report. However, the report stated that based on the sponsor’s and CBER analyses of serology results the animals were found to be suitable for use in the study.

Blood samples for culture, CBC, coagulation battery, and clinical chemistry were collected pre-challenge and daily thereafter. Additional blood samples for culture were obtained from animals that had fever (>1.5C increase over baseline for 2 consecutive measurements) beyond Day 4 and

when the animal was determined to be moribund or dead. Necropsies were performed on animals that succumbed to challenge. Any respiratory secretions were cultured. Lungs, intrathoracic lymph nodes, and any gross lesions were examined microscopically.

Study Results

Table 13 Aerosol Exposure by Animal

Animal ID	Sex	Weight (kg)	Estimated inhaled dose (CFUs)	Number of LD50 equivalents
X486	M	4.3	1.46×10^4	43
X753	F	3.2	2.59×10^4	76
Y256	M	4.8	3.00×10^4	88
X900	M	5.1	1.25×10^4	37
Y213	F	3.3	2.58×10^4	76
Y212	F	3.5	1.21×10^4	36
X950	M	4.7	0.866×10^4	25
W904	F	3.3	1.28×10^4	38
X603	F	3.4	1.29×10^4	38
X840	M	5.2	0.825×10^4	24
Mean±SD		4.1±0.8	$1.64 \times 10^4 \pm 0.78 \times 10^4$	48.1±23
Median		3.9	1.29×10^4	38

Modified from the study report

MO comment: Target $\times 100$ LD50 dose was closely approximated only in 1 animal (Y256). The rest of the animals were exposed to doses lower than target. Exposure in 2 animals was at 25% of the target. Nevertheless, all animals challenged in this study succumbed to disease. Therefore, it appears that challenge dose of $\times 24$ LD50 (8.25×10^3) are lethal in this animal model. Targeting $\times 100$ LD50 is likely to result in uniform mortality in the untreated controls. It will be important to examine the correlation between the exposure dose, appearance of symptoms of disease, and the survival time.

Clinical Signs

The average time to the onset of fever, defined as sustained temperature elevation of ≥ 1.5 C above baseline for 2 hours, was 54.3 h post-challenge (range 47 h to 61.5 h) and the fevers lasted until the animals succumbed to infection. The average time until AGM succumbed to infection following onset of fever was 39.2 h (range 17.5 h to 78 h). There was not a statistically significant relationship between the aerosol dose and the interval of time between onset of fever and death.

Table 14 Fever Onset

Animal ID	Time from challenge to fever onset (h)	Time of fever onset (date and h)	Time of challenge (date and h)	Temperature at onset of fever (C)	Baseline average temperature (C)	Target temperature for fever (baseline+1.5C) (C)
X486	58	1/7/09 22:00	1/5/09 12:00	39.1	37.1	38.6
X753	61.5	1/8/09 02:00	1/5/09 12:23	38.7	36.7	38.2
Y256	48	1/7/09 18:00	1/5/09 14:50	40.1	38.1	39.6

X900	61	1/8/09 02:00	1/5/09 13:02	38.7	36.9	38.4
Y213	56.5	1/7/09 22:00	1/5/09 13:26	38.7	36.9	38.4
Y212	55	1/7/09 21:00	1/5/09 13:50	38.5	36.8	38.5
X950	50	1/7/09 16:00	1/5/09 14:05	39.7	38	39.5
W904	54.5	1/7/09 21:00	1/5/09 14:30	38.9	37	38.5
X603	51	1/7/09 18:00	1/5/09 14:50	39.6	37.6	39.1
X840	47	1/7/09 14:00	1/5/09 15:08	39.8	37.8	39.3

MO comment: Incubation period (time to fever onset) on average lasted 55 hours. As compared to the previous study there appears to be slightly longer duration of incubation period, that might be attributable to lower average exposure dose administered. As in the previous study the blood for bacteremia was collected on the daily basis. The correlation between the fever onset and bacteremia therefore cannot be established. All animals became febrile postexposure. There seems to be no correlation between the estimated challenge dose and the timing of sustained elevated temperature.

Telemetry Findings other than Fever

A significant increase in the heart rate was observed in all animals on 2-3 day post-challenge corresponding to the onset of fever. This was accompanied by some increase in blood pressure. Progression of the disease was accompanied by the drop in systolic and diastolic pressures. Changes in respiratory function also closely followed fever onset and manifested by respiratory rate increase and shallow breathing.

MO comment: This findings represent overall inflammatory response to infection. Changes in respiratory rate could also be a consequence of parenchymal lung disease. No radiologic examinations were performed in this study, thus the onset of respiratory distress as evident by tachypnea cannot be directly correlated to the lung infiltrate development. However, inflammatory changes as well as the finding of hemorrhage and necrosis on pathological examination of lungs in all animals confirm the disease as pneumonic plague.

Table 15 Signs and Symptoms Post Challenge

	ID	X486	X753	Y256	X900	Y213	Y212	X950	W904	X603	X840
Date	Sex	M	F	M	M	F	F	M	F	F	M
1/06/2009	6 am	N	NE	N	N	NE	NE	N	NE	NE	N
	2 pm	H	N	N	H	N	N	N	NE, H	N	N
	10 pm	N	N	N	N	N	N	N	H	N	N
1/07/2009	6 am	H	NE	N	H, NE	NE	NE	N	NE, H	NE	N
	2 pm	H	NE	N	H	N	NE, H	NE	NE, H	N	N
	10 pm	H	H	H	N	H	H	H	H	N	N
1/08/2009	6 am	H, L	NE, L	NE, L, C, P	H, L	NE, H, L	NE, H	NE, H, L	NE, H, L	NE, H	NE
	2 pm	H, L, NE, BND	NE, L	FD (8:30)	NE, H	NE, H, L	NE, H, L	NE, H, L, NS	NE, H, L, NS	NE, H, NS	NE, H
	10pm	H, L, PS, BND	H, L		H, NS	FD (19:00)	FD (16:30)	H, L	NS, H, L	H, L, NS	H, L
1/09/2009	6 am	FD (3:30)	H, NE, L		H, L, NE			FD (3:00)	NE, H, L	NE, H, L, NS	NE, H

	ID	X486	X753	Y256	X900	Y213	Y212	X950	W904	X603	X840
Date	Sex	M	F	M	M	F	F	M	F	F	M
	2 pm		NE, L, H, NS, SA, RD		NE, H, L, NS				NE, H, L, NS	FD (7:00)	NE, H, L, RD, OB
	10 pm		FD (19:00)		H, L, BND				H, L, NS		FD (19:30)
1/10/2009	6 am				NE, H, L				NE, H, L, NS, RD		
	2 pm				NE, NS, H, L				FD (7:00)		
	10 pm				H, L, NS						
1/11/2009	6 am				NE, H, NS, L, BND, RD						
					FD (8:00)						

BDM = Bloody mouth discharge
N = Normal
BND = Bloody nasal discharge
NE = Not eating
FD = Found dead

NS = No stool
H = Hunched posture
L = Lethargic
OB = open mouth breathing
RD = Respiratory distress

P = prostrate
SA = salivation
C = coughing
FD = found dead

MO comment: A combination of hunched posture and loss of appetite appear to be a consistent finding on Day 2 post challenge among all animals. However in some animals these signs were followed by onset of death shortly thereafter (~8 hours), while some others were able to survive for > 60 hours after onset of these symptoms. The fever appears to be a more reliable sign of the illness onset. The limitation of the study in identifying a reliable trigger for intervention is infrequent blood cultures (once daily). It precludes a correlation of a symptom, sign, laboratory abnormality to the presence of bacteria in the blood.

Laboratory abnormalities

Several of the post-challenge hematological parameters were indicative of an active infection. On post-challenge Day 3 7/9 surviving AGMs developed neutrophilic leukocytosis. Other notable findings on CBC included basophilia, and eosinophilia.

Table 16 Hematologic Parameters, Changes from Baseline, Study Day

Hematology Parameter	Study Day 1		Study Day 2		Study Day 3		Study Day 4	
	N	Mean Shift						
Red Blood Cell Count [‡]	10	1.022	10	0.982	9	0.932 ↓	4	0.942
Hemoglobin	10	0.330	10	-0.100	9	-0.700	4	-0.550
Hematocrit [‡]	10	1.055 ↑	10	0.998	9	0.931 ↓	4	0.947
MCV	10	2.510 ↑	10	1.160 ↑	9	-0.133	4	0.325
MCH [‡]	10	1.004	10	1.008 ↑	9	1.010	4	1.010
MCHC [‡]	10	0.971 ↓	10	0.992	9	1.010	4	1.005
RDW [‡]	10	0.994	10	0.985 ↓	9	1.003	4	0.992
Platelet Count [‡]	10	1.123 ↑	10	1.116 ↑	9	0.947	4	0.691 ↓
MPV [‡]	10	1.075 ↑	10	1.051 ↑	9	1.057	4	1.066
White Blood Cell Count [‡]	10	1.137	10	1.171	9	2.441 ↑	4	1.757 ↑
Neutrophils [‡]	10	0.662 ↓	10	0.736 ↓	9	3.230 ↑	4	2.238 ↑
Lymphocytes	10	1.999 ↑	10	2.117 ↑	9	1.622	4	0.648
Neutrophils/Lymphocytes Ratio [‡]	10	0.368 ↓	10	0.402 ↓	9	2.350 ↑	4	1.963 ↑
Monocytes	10	0.019	10	-0.010	9	0.108	4	0.120
Eosinophils [‡]	10	1.537	10	1.691 ↑	9	3.017	4	1.155
Basophils [‡]	10	1.236	10	1.389	9	3.980 ↑	4	1.732

‡ Distribution was log-normal for this parameter.

↑, ↓ Indicates that, for untransformed data, the difference between baseline and study day means was significantly different from zero (at the 0.05 level); for log-transformed data, the ratio of geometric means at baseline and study day was significantly different from 1 (at the 0.05 level). “↑” indicates that the mean at the study day was greater than baseline, while “↓” indicates that the mean at the study day was less than baseline.

No significant changes were noted on examination of clinical chemistry results other than LFTs (AST) and RFT (Cr) elevation in animals surviving to Day 3 post challenge.

Table 17 Serum Chemistry, Changes from Baseline, Study Day

Clinical Chemistry Parameter	Study Day 1		Study Day 2		Study Day 3		Study Day 4	
	N	Mean Shift						
Total Bilirubin [‡]	10	0.630 ↓	10	0.588 ↓	9	0.580 ↓	4	1.078
Aspartate Aminotransferase [‡]	10	1.249 ↑	10	1.032	9	2.474 ↑	4	1.554 ↑
Alanine Aminotransferase [‡]	10	1.159 ↑	10	1.145 ↑	9	1.170 ↑	4	1.179
Lactate Dehydrogenase [‡]	10	1.140	10	1.014	9	2.271 ↑	4	1.892 ↑
Total Protein [‡]	10	1.043 ↑	10	1.024	9	1.030	4	1.057
Blood Urea Nitrogen	10	6.180 ↑	10	2.480	9	5.689 ↑	4	8.825
Creatinine	10	0.282 ↑	10	0.229 ↑	9	0.346 ↑	4	0.420 ↑
Sodium [‡]	10	1.030 ↑	10	1.028 ↑	9	1.017 ↑	4	1.019
Potassium [‡]	10	1.032	10	1.032	9	0.941	4	0.831 ↓
Chloride [‡]	10	0.957 ↓	10	0.966 ↓	9	0.983	4	0.972 ↓
Calcium	10	1.185 ↑	10	1.104 ↑	9	0.334 ↑	4	0.305
Phosphorus	10	1.214 ↑	10	0.974	9	-0.588	4	-0.728

‡ Distribution was log-normal for this parameter.

↑, ↓ Indicates that, for untransformed data, the difference between baseline and study day means was significantly different from zero (at the 0.05 level); for log-transformed data, the ratio of geometric means at baseline and study day was significantly different from 1 (at the 0.05 level). "↑" indicates that the mean at the study day was greater than baseline, while "↓" indicates that the mean at the study day was less than baseline.

Analysis of coagulation parameters showed that most animals (90%) had coagulation abnormalities: elevated PT and PTT along with increased fibrinogen).

Table 18 Coagulation Parameters, Changes from Baseline, Study Day

Coagulation Parameter	Study Day 1		Study Day 2		Study Day 3		Study Day 4	
	N	Mean Shift						
Prothrombin Time [‡]	10	1.019	10	1.048 ↑	9	1.287 ↑	4	1.129
Fibrinogen Concentration [‡]	10	1.257 ↑	10	1.300 ↑	9	1.711 ↑	4	3.512 ↑
Activated Partial Thromboplastin Time [‡]	10	1.041	10	1.045 ↑	9	1.439 ↑	4	1.558 ↑
D-Dimer [‡]	10	0.483 ↓	10	0.805	9	0.481 ↓	4	0.573

‡ Distribution was log-normal for this parameter.

↑, ↓ Indicates that the ratio of geometric means at baseline and study day was significantly different from 1 (at the 0.05 level). "↑" indicates that the mean at the study day was greater than baseline, while "↓" indicates that the mean at the study day was less than baseline.

MO comment: Again changes in laboratory parameters were noted in 100% of animals. As the samples were collected on the daily basis, the correlation of the abnormalities to the onset of fever or bacteremia could not be established. Acute inflammatory response to infection was prominent in most animals studied.

Table 19 Quantitative Bacteremia (cfu/ml)

Animal ID	Challenge Dose (cfu)	Day 1	Day 2	Day 3	Day 4	Day 5	Terminal
X486	1.46×10^4	0	+	2.63×10^6			8.03×10^7
X753	2.59×10^4	0	0	8.80×10^3	+2		7.43×10^6
Y256	2.98×10^4	0	2.69×10^3				1.03×10^9
X900	1.25×10^4	0	0	1.70×10^3	4.97×10^3	1.70×10^4	8.63×10^6
Y213	2.58×10^4	0	0	2.80×10^6			2.41×10^8
Y212	1.21×10^4	0	0	9.93×10^6			4.73×10^7
X950	8.66×10^3	0	2.68×10^3	9.03×10^7			1.16×10^9
W904	1.28×10^4	0	0	6.23×10^2	1.36×10^3		3.43×10^5
X603	1.29×10^4	0	+	7.40×10^6			2.16×10^9
X840	8.25×10^3	0 ₃	6.13×10^2	1.46×10^3	9.07×10^4		7.23×10^7

1 0 = negative for *Y. pestis*; + = positive for *Y. pestis*, but <250 cfu/mL.

2 This blood sample probably contained >250 cfu/mL. Due to the condition of the blood, a sample within the range for enumeration (250-3000 cfu/mL) could not be assayed.

3 A presumptive positive result could not be confirmed upon further investigation.

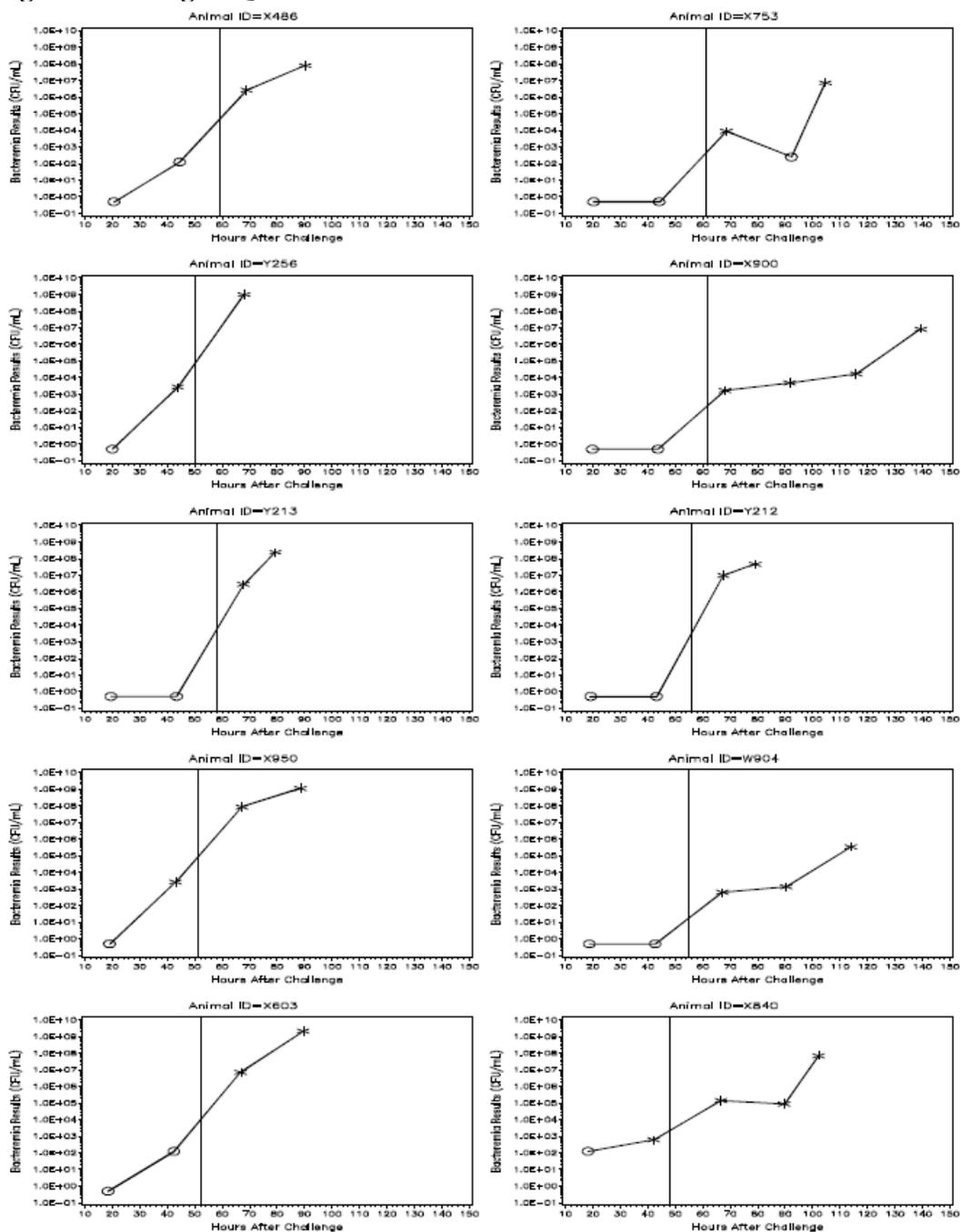
Modified from the study report

MO comment: For all animals after bacteremia onset, subsequent cultures showed substantial increase in cfu of *Y. pestis* until terminal culture. There was no correlation between the challenge dose and the onset, degree, or duration of subsequent bacteremia. The below time plot of quantitative cultures shows that even with sparse blood culture sampling, 50% of animals were bacteremic prior to and at the onset of fever. It is possible therefore that animals found to be febrile are in fact bacteremic at the same time.

As presented during NIAID plague animal model annual update on 02/24/2011 average time to first positive blood culture was 51 hours (range 30-68.5h).

Animal ID	Challenge Dose (cfu)	Challenge Time (date, h)	First Positive Blood Culture Time (date, h)	Time to Positive Blood Culture (h)
X486	1.46×10^4	1/5/09 12:00	1/7/09 8:00	44
X753	2.59×10^4	1/5/09 12:23	1/8/09 9:00	68.5
Y256	2.98×10^4	1/5/09 14:50	1/7/09 8:00	41
X900	1.25×10^4	1/5/09 13:02	1/8/09 9:00	68
Y213	2.58×10^4	1/5/09 13:26	1/8/09 8:00	41.5
Y212	1.21×10^4	1/5/09 13:50	1/8/09 9:00	43
X950	8.66×10^3	1/5/09 14:05	1/7/09 9:00	43
W904	1.28×10^4	1/5/09 14:30	1/8/09 9:00	66.5
X603	1.29×10^4	1/5/09 14:50	1/7/09 9:00	66
X840	8.25×10^3	1/5/09 15:08	1/6/09 9:00	30
Mean±SD	$1.64 \times 10^4 \pm 0.78 \times 10^4$			51.2±14.4
Median	1.29×10^4			43.5

Figure 2 Timing of Quantitative Bacteremia Relative to Fever Onset



The vertical line in each plot indicates the time of onset of fever in each AGM.
 O Negative results plotted at 0.5 CFU/mL (1/2 of 1), '+' results plotted at 125 CFU/mL (1/2 of 250),
 '-2+' result (at least 250 CFU/mL) plotted at 250.
 * Reportable values.

Adapted from the study report

MO comment: Based on the graph analysis it appears likely that most animals will be found to be bacteremic if the cultures were to be drawn right around time of fever onset.

Other microbiology results

Samples of fluids were obtained from the nasal cavity and trachea of all AGMs during necropsy. All of these samples yielded positive cultures of *Y. pestis*.

MO comment: Individual animal data on microbiology results for cultures other than blood were not provided in this report or accompanying datasets.

Timing of death (TOD)

Respiratory rate recordings were used as the primary criteria for TOD determinations based on telemetry data. When respiratory rate values decreased and were no longer recorded, a “No Data” message was produced, which was an indicator death. If respiratory data were not available or did not clearly indicate a TOD, temperature was used as the secondary parameter. TOD obtained from temperature was determined when the temperature was < 36°C. Blood pressure recordings < 5 mmHg were also used to support TOD determinations. Telemetry blood pressure values are calculations equal to the average of systolic and diastolic blood pressures for each 30 sec time interval.

In this study animals survived to 67-139 h post challenge.

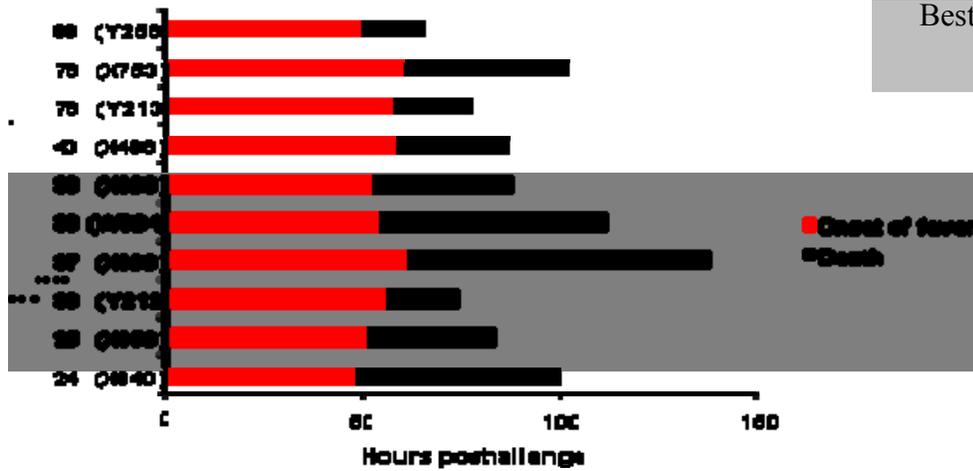
Table 20 Time to Onset of Fever and Time to Death

Animal ID	Sex	Estimated inhaled dose (CFUs)	Time to onset fever (h post-challenge)	Time from onset of fever to death (h)	Time to death (h post-challenge)
X486	M	1.46×10^4	58	29.5	87.5
X753	F	2.59×10^4	61.5	43	104.5
Y256	M	3.00×10^4	48	19.5	67.5
X900	M	1.25×10^4	61	78	139
Y213	F	2.58×10^4	56.5	21	77.5
Y212	F	1.21×10^4	55	17.5	72.5
X950	M	0.866×10^4	50	35	85
W904	F	1.28×10^4	54.5	58	112.5
X603	F	1.29×10^4	51	37	88
X840	M	0.825×10^4	47	53.5	100.5
Mean±SD		$1.64 \times 10^4 \pm 0.78 \times 10^4$	54.3±5.1	39.2±19.4	93.5±21.4
Median		1.29×10^4	54.8	36	87.8

MO comment: Most animals succumbed to death within 38 hours of fever onset. Three animals in this study lived well under 24 hours after fever onset, reaffirming the need for early intervention in some animals. As in the previous study the blood for bacteremia was collected on the daily basis. The correlation between the fever onset and bacteremia therefore cannot be established. There is a 100% correlation between onset of fever and subsequent death; all animals were febrile on the study postexposure and all succumbed to death. There seems to be no correlation between the estimated challenge dose and the timing of sustained elevated temperature or the onset of death. The graph below from the study report confirms just that.

Figure 3 Onset of fever and time to death relative to aerosol dose exposure

Battelle Study 878



Adapted from the Battelle presentation at the annual NIAID plague animal model update held on 02/24/2011

MO comment: This graphical presentation of onset of fever, defined as temperature $\geq 1.5^{\circ}\text{C}$ above baseline and sustained for >2 hours, and death was provided by Battelle during NIAID plague animal model update on 02/24/2011. Onset of fever and time to death did not correlate with the challenge dose (Y axis of the above graph) administered in this study.

Pathology

All infected monkeys died prior to study termination on Day 21 and underwent complete necropsies. Protocol specified tissue samples (lungs and bronchi, bronchial and mediastinal lymph nodes and gross lesions) were examined microscopically.

A summary table below provides the findings from gross pathology examination of the study animals.

Table 21 Gross pathology results

Sex	M	F
Mortality (%)	100%	100%
ORGAN/lesion # dead	5	5
BRAIN Discoloration	1	0
CAVITY, PERICARDIAL Fluid	1	1
CAVITY, THORACIC Fluid	5	4
LYMPH NODE, BRONCHIAL Enlarged/Discoloration	2	1
LYMPH NODE, MANDIBULAR Discoloration	1	0
LYMPH NODE, MEDIASTINAL Enlarged	2	2
LYMPH NODE, OTHER Enlarged/Discoloration	1	1
LUNG Focus/Discoloration/ Nodule/Mass	5	4
LUNG Fluid	1	0
LUNG Adhesion	0	1
NOSE/TURBINATES Fluid	1	0
SPLEEN Discoloration	1	1
THYMUS Fluid	1	1

Adapted from the study report

On microscopic examination all animals had inflammatory changes in the lungs and thoracic lymph nodes with *Y. pestis* identified in the alveoli, bronchial spaces, and bronchial lymph nodes. Two animals had hemorrhagic changes of the spleen and two of the thymus. All four of these animals also had *Y. pestis* identified on the microscopy. One animal had hemorrhagic meningitis.

Table 22 Histopathology findings

ID	X486	Y256	X900	X950	X840	X753	Y213	Y212	W904	X603
Tissue/Sex	M	M	M	M	M	F	F	F	F	F
Death	Found dead, D4	Found dead, D3	Found dead, D6	Found dead, D4	Found dead, D4	Found dead D4	Found dead D3	Found dead D3	Found dead D5	Found dead D4
LN bronchial	bacteria ++, necrosis ++, edema +	bacteria +++, necrosis +++, edema +, hemorrhage +, fibrin ++	bacteria +++, hemorrhage ++, fibrin +++, inflammation +, necrosis +++++	bacteria +++, necrosis +++++, hemorrhage ++, edema ++, fibrin ++	bacteria ++, necrosis ++, hemorrhage ++, fibrin ++, inflammation +	bacteria +++, hemorrhage +, necrosis +++, fibrin ++, inflammation +	bacteria ++, necrosis ++, inflammation +, edema ++	bacteria ++, fibrin +, edema ++, necrosis +	bacteria ++, necrosis +++, fibrin +, hemorrhage +	bacteria +++, necrosis +++, edema ++, hemorrhage +
LN mediastinal	bacteria +++, edema++, necrosis +	bacteria +, hemorrhage +	bacteria ++, hemorrhage ++, fibrin ++	bacteria ++, necrosis+, hemorrhage ++, fibrin +,	bacteria +, necrosis +++, hemorrhage ++	bacteria ++, hemorrhage +, necrosis +++, fibrin ++,	bacteria +++, necrosis ++	bacteria ++, necrosis ++	bacteria ++, necrosis +++, fibrin +, hemorrhage +	bacteria +, necrosis +, hemorrhage +, inflammation +
Lung	bacteria ++, hemorrhage ++, fibrin +, alveolar macrophage hyperplasia ++	bacteria ++, alveolar macrophage hyperplasia +, inflammation +, hemorrhage +, fibrin +	bacteria +++, hemorrhage +++++, inflammation +++++, necrosis +++++, pleural fibrin +	bacteria ++, hemorrhage +, alveolar macrophage hyperplasia +	bacteria +++++, inflammation +++, edema +++, hemorrhage +++++, fibrin +++++, necrosis +++++	bacteria +++, inflammation +++, necrosis ++, fibrin ++, hemorrhage +++, inflammation ++	bacteria ++, alveolar macrophage hyperplasia +, fibrin+	bacteria +, hemorrhage ++, fibrin+, inflammation ++, alveolar macrophage hyperplasia +	bacteria +++++, hemorrhage +++, fibrin +++++, inflammation +++++, necrosis +++++	bacteria ++, edema ++, fibrin ++, hemorrhage ++, fibroplasia +, inflammation ++, alveolar macrophage hyperplasia +
Spleen	unremarkable	unremarkable	bacteria +, necrosis ++, hemorrhage +	unremarkable	unremarkable	unremarkable	unremarkable	unremarkable	unremarkable	bacteria +++++, necrosis ++, hemorrhage +
Thoracic cavity	red fluid 40 ml	red fluid 60 ml	viscous dark fluid 10 ml	red fluid 30 ml	red fluid 25 ml	red fluid 30 ml	clear fluid 30ml	clear fluid 15 ml	unremarkable	viscous dark fluid 25 ml, adhesions
Heart	unremarkable	pericardial clear fluid 10ml	unremarkable	unremarkable	unremarkable	unremarkable	unremarkable	unremarkable	unremarkable	red pericardial fluid 8ml
Thymus	bacteria +, edema ++, hemorrhage +	unremarkable	unremarkable	unremarkable	unremarkable	unremarkable	unremarkable	unremarkable	unremarkable	bacteria +, edema ++, hemorrhage +, necrosis +
Other tissues	Mandibular LN: bacteria +++++, hemorrhage+, necrosis +, edema +	unremarkable	Inguinal LN: Bacteria +, lymphoid hyperplasia ++	Brain: bacteria ++, fibrin +, hemorrhage ++	Nasal turbunates clear fluid 5ml	unremarkable	unremarkable	unremarkable	unremarkable	Lumbar LN: bacteria +++, hemorrhage++, lymphoid hyperplasia ++

MO comment: *The pathology report findings of fibrinosuppurative pneumonia are consistent with pneumonic plague in all of the animals.*

Conclusions:

The study results demonstrate that the inhalational model of pneumonic plague in AGM is uniformly fatal at exposures of >24 LD₅₀ (8.25×10^3). All animals develop elevated temperature response ($>1.5^\circ\text{C}$ over baseline sustained for >2 hours) to the infection approximately 55 hours post exposure and then succumb to the disease 35 hours later. High fever ($>40^\circ\text{C}$), loss of appetite, and hunched posture are the most consistent signs of the disease in this study. Animals developed tachycardia and tachypnea at the time of temperature elevation. Laboratory abnormalities observed most often included neutrophilic leukocytosis, elevation of liver enzymes and creatinine. Coagulation abnormalities were also common. All animals were found to be bacteremic as early as Day 2 post challenge; in 5 of them positive blood culture preceded onset of fever. Respiratory secretions collected at the time of necropsy in all animals were found to be positive for *Y. pestis*. Upon necropsy/histopathology examination changes in lungs, mediastinal lymph nodes were characteristic of pneumonic plague. The pathogen, route of exposure, clinical signs and symptoms, course of the disease as well as laboratory abnormalities and histopathology findings are fairly similar between AGM and humans with pneumonic plague. Selection of animals for treatment based on fever (trigger for intervention) is likely to result in a substantial number of animals bacteremic at the time of treatment administration and will allow for efficacy analyses to be conducted on a more conservative (microbiologically confirmed plague) animal population.

Study report “A Natural History Study of Inhalational Plague *Y. pestis* Strain CO92 in Adult Telemetered African Green Monkey”, Lovelace Respiratory Research Institute (LRRRI) Study Protocol FY06-126 (NIAID protocol D13-1)

The study was not Good Laboratory Practices (21 CFR Part 58) compliant. The study was conducted according to LRRRI standard operating procedures (SOPs) and study-specific procedures (SSPs). This study was conducted in LRRRI's Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facility.

Animals

Twelve specific pathogen-free (TB, SIV, SRV, STLV, SHF, HBV), symptom and malformation free wild caught AGMs (*Chlorocebus aethiops*) were provided by NIH for this study. Ten (5 male, 5 female) AGMs weighing 3-6 kg were placed on the study. Two animals: 1 male and 1 female AGMs were available as replacements prior to the day of challenge. Animals were initially quarantined for 30 days, then surgically implanted telemetry devices and Broviac catheters and allowed to recover for 14 days post surgery. Animals were identified by tattoos and individual cage cards. Post operative Pen G was administered to all animals for 5 days.

MO comment: No screen for prior exposure to environmental Yersiniae and Y. pestis was performed.

Challenge Agent

Yersinia pestis strain CO92 was the challenge agent in this study. Organism was administered via nebulized aerosol in the head-only exposure chamber. Animals were divided in two groups of 5 were anesthetized and challenged on April 2, 2007 and on April 23, 2007. AGMs were anesthetized, placed into the plethysmography chamber, and challenged with aerosolized *Y. pestis* strain CO92. The inoculum consisted of suspensions of freshly grown bacteria prepared and aerosolized using Battelle SOP Number BBRC.X1444 and BBRC.XIII-001. Aerosol samples were collected during each exposure by using sterile impingers containing 20 ml of sterile phosphate buffered saline, gelatin, with a pH of 7.0 +/- 0.5. The duration of the aerosol challenge was based on an estimated aerosol CFU concentration and a cumulative minute volume measured during the challenge.

Table 23 Study Schedule

Procedure	Week -2 to Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
<i>Y. pestis</i> challenge		X								
Arterial blood gas ^{a,b}	X		X ^b	X ^b	X ^b	X ^b	X ^b			
Thoracic radiograph ^c		X	At interim, at increased respiration, and before terminal necropsy							
Test article infusion (Q12h)			X	X	X	X	X	X	X	X
Quantitative bacteriology ^a	X		X	X	X	X				
Serum chemistry ^a	X		X	X	X	X				
Coagulation indices ^a	X		X	X	X	X				
Hematology ^a	X		X	X	X	X				
Tissue pathogen load ^a			All dead or euthanized monkeys							
Body weight ^d	X	X								X
Telemetry	X	X	X	X	X	X	X	X	X	X
Twice daily detailed clinical observations		X	X	X	X	X	X	X	X	X
Interim necropsy ^a			Dead or moribund monkeys							
Terminal necropsy ^a										X

^aFor animals that were alive on Days 1–4, daily blood samples were collected for quantitative bacteriology, serum chemistry, coagulation indices, and hematology. At interim and terminal necropsies (1) blood was collected for arterial blood gas determination, quantitative bacteriology, serum chemistry, coagulation indices, and hematology, and (2) tissues were obtained for pathogen load.

^bArterial blood gases were measured as frequently as daily after infection over Days 1–5 based on clinical observations and at necropsy.

^cThoracic radiographs were taken in anesthetized animals prior to *Y. pestis* challenge on Day 0, at increased respiration based on telemetry, and just before terminal necropsy.

^dBody weight also was obtained on the day of necropsy.

^eTerminal necropsies were performed on any surviving animals on Day 8 post challenge.

Study Results

Exposure Dose

Inhaled aerosol dose was measured using a plethysmography, where respiratory rate, tidal volume and minute volume were recorded. The exposure was targeted at 100x LD50 (~100x cfu of *Y. pestis*)². Quantitative cultures were taken from these impinger samples to confirm the bacterial concentrations of the aerosolized inoculum. The actual pathogen dose was ultimately determined by the concentration of bacteria in the nebulizer suspension, sample time/exposure time, the AGMs tidal volume, and the spray factor (the ratio of the aerosol concentration to the nebulizer concentration). Please refer to Clinical Microbiology review by Simone Shurland and Pharmacology Toxicology review by Stephen Hundley for the detailed assessment of the stock pathogen, aerosol procedures, and calculation of the challenge doses for individual animals.

Table 24 Aerosol exposure to *Y. pestis* CO92 by Study Animal

Animal ID	Sex	Weight (kg)	Estimated inhaled dose (cfu)	LD50 equivalent
X666	F	3.5	7.21×10^4	206
X705	F	4.1	5.60×10^4	160

² Adamovicz, J. J., and Worsham, P.L. (2006). Plague in *Biodefense research methodology and animal models*, J. R. Swearingen, Ed. (CRC Press, Boca Raton, FL, 2006), chapter 8, pp. 107-135.

Animal ID	Sex	Weight (kg)	Estimated inhaled dose (cfu)	LD50 equivalent
X756	M	5.3	8.94×10^4	255
X532	M	5.5	5.71×10^4	163
X538	M	6.5	5.85×10^4	167
Cohort 1 Mean±SD		5.0±1.2	$6.66 \times 10^4 \pm 1.43 \times 10^4$	190±41
X775	F	4.4	2.58×10^4	74
X784	M	4.6	3.19×10^4	91
X789	M	3.5	4.51×10^4	129
X790	M	3.5	1.54×10^4	44
X774	F	4.0	2.02×10^4	58
Cohort 2 Mean±SD		4.0±0.5	$2.77 \times 10^4 \pm 1.15 \times 10^4$	79±33
Cohort 1&2 Mean±SD		4.5±1.0	$4.71 \times 10^4 \pm 2.39 \times 10^4$	134±68
Median		4.2	5.10×10^4	144

MO comment: As the exposures were higher than target in the first group of challenged animals, adjustments to nebulizer settings were made.

Clinical Observations

From the day of exposure (Day 0), the following parameters were monitored twice daily: activity, posture, nasal discharge, sneezing, coughing, respiratory character, ocular discharge, anorexia, stool characteristics, seizure, neurologic signs, and other abnormalities. Table 22 summarizes these clinical observations.

Table 25 Clinical Observations by Study Day (Sign/Symptom onset)

Animal No.	Hunched	Nasal Discharge	Decrease in Appetite	Decrease in Activity	Respiratory Character	Abnormal Stool	Neurological Signs
X666	1	–	1	–	3	3	2
X705	2	–	1	2	–	2	2
X756	3	–	1	2	2	2	2
X532	3	–	1	1	3	2	2
X538	1	–	1	1	2	2	2
X774	3	3	2	3	3	2	3
X775	–	3	2	–	4	1, 4	–
X784	3	–	3	3	3	2	4
X789	2	–	2	0	3	0	–
X790	3	–	1	2	–	1, 3	–

Adapted from the study report

MO comment: Animals in 1st (higher dose cohort) appear to have manifested their disease in more uniform manner as compared to low dose cohort. Decrease in appetite followed by decrease in activity, abnormal stools and neurological signs. Onset of respiratory distress and hunched posture occurred later as disease progressed in most animals.

Telemetry

Temperature. Temperature was recorded every minute and averaged for by hour. Average pre-exposure temperature minimum at 02:00–04:00 was 36.5 with a range from 35.1 to 37.3°C.

Average pre-exposure temperature maximum between 14:00–18:00 was 37.5 with ranges from 36.9 to 38.3°C. All animals had a post-exposure temperature maximum of 39°C, but this temperature elevation was not sustained in all animals. The average time to first temperature elevation over 39°C was 67 ±11 hr.

Appearance of 39°C for two consecutive hours was selected as the onset of fever.

Physiologically significant tachycardia, defined either as a heart rate greater than 200 beats per minute (bpm) or an increase in 50 bpm over baseline was seen in all but one animal. Respiratory rate over 40 breaths per minute (brpm) was consistently seen in all animals.

In some animals the onset of fever, tachycardia and tachypnea coincided, although fever more often preceded the other two signs by 2 to 3 hr. The onset of each sign of the disease varied somewhat between animals.

Table 26 Onset of Fever, Tachycardia and Tachypnea in Challenged Animals

Animal #	Temperature			Heart Rate		Respiratory Rate		
	>39°C	>1°C ↑ ^a	>2°C ↑	>200b/m ^b	>50b/m ↑	40br/m ^c	>5br/m ↑	>10br/m ↑
532	83	74	77	73	75	73	73	75
538	73	67	73	75	68	50	57	57
666	73	69	72	72	80	68	75	80
705	51	46	47	53	53	IR# ^d	IR# ^d	IR# ^d
756	67	61	64	NA ^e	65	73	65	66
775	74.5	73.5	78.5	76.5	76.5	79.5	72.5	79.5
774	51	50	51	51	51	53	51	52
784	33.3	29.3	32.3	32.3	32.3	32.3	29.3	32.3
789	74.5	74.5	80.5	74.5	81.5	73.5	69.5	73.5

^a ↑ = Increase over baseline; ^b b/m = beats per minute; ^c br/m = breaths per minute; ^d = #IR = inadequate recording; ^e NA=not achieved

Adapted from study report

MO comment: An increase in the heart rate was observed in all animals beginning approximately 2-3 days post-challenge. This was accompanied by a slight but sustained increase in respiratory rate and fever. Body temperature demonstrated diurnal variation from 35 to nearly 39 °C prior to challenge and then increased to a sustained temperature of > 40 °C approximately 1.5-2 day post challenge.

Table 27 Baseline Average Temperature (C)

Animal ID/ Hour	X666	X705	X756	X532	X538	X775	X784	X789	X790	X774
0	36.3	36.0	35.9	36.0	36.4	36.7	36.0	37.0	36.3	36.3
1	36.2	36.0	36.0	36.0	36.4	36.6	36.1	36.9	36.2	36.2
2	36.1	36.0	36.0	36.0	36.5	36.7	36.1	36.8	36.2	36.2
3	36.0	36.0	36.0	36.0	36.5	36.7	36.1	36.7	36.2	36.2
4	35.8	35.9	36.0	35.9	36.5	36.8	36.1	36.6	36.3	36.2
5	35.6	35.8	35.9	35.8	36.4	36.8	36.0	36.5	36.3	36.3
6	35.1	35.8	36.0	35.8	36.4	36.9	36.1	36.4	36.3	36.5
7	35.9	35.8	35.9	35.8	36.4	37.3	36.3	36.7	36.5	36.8
8	36.0	35.8	36.1	35.8	36.5	37.6	36.5	37.1	36.8	37.1

9	35.9	35.9	36.7	35.9	37.0	38.3	37.1	37.7	37.4	37.5
10	36.1	36.3	36.9	36.3	37.3	38.4	37.3	37.9	37.7	37.6
11	36.3	36.3	36.9	36.3	37.1	38.3	37.0	37.9	37.4	37.6
12	36.6	36.3	36.8	36.3	36.9	38.0	36.8	37.9	37.4	37.2
13	36.8	36.4	36.6	36.4	37.0	37.8	36.8	37.9	37.0	37.0
14	36.9	36.6	36.8	36.6	37.2	37.8	37.0	37.9	37.2	37.1
15	37.3	36.2	37.0	36.2	37.3	38.0	37.1	37.7	37.4	37.3
16	37.4	36.4	36.9	36.4	37.3	37.9	36.9	37.9	37.2	37.2
17	37.4	36.5	36.8	36.5	37.4	37.8	36.9	38.0	37.2	37.1
18	37.2	36.5	36.4	36.5	37.2	37.5	36.9	38.0	37.2	36.9
19	36.9	36.4	36.1	36.4	37.1	37.1	36.5	37.7	36.9	36.6
20	36.8	36.1	36.0	36.1	36.7	37.0	36.2	37.6	36.5	36.4
21	36.6	36.0	36.0	36.0	36.5	36.8	36.0	37.4	36.4	36.4
22	36.4	36.0	35.9	35.9	36.5	36.8	36.0	37.3	36.3	36.3
23	36.4	35.9	35.9	35.9	36.4	36.7	36.0	37.1	36.3	36.4

Table 28 Fever Onset (FDA definition >1.5 C above baseline for >2h)

	Time from challenge to fever onset (h)	Time of fever onset (date and h)	Time of challenge (date and h)	Temperature at onset of fever (C)	Baseline average temperature (C)	Target fever temperature (baseline+1.5C) (C)	Episodes of no fever
X666	67	4/5/07 5:00	4/2/07 9:58	37.9	35.6	37.1	1x 4h ptd
X705	46	4/4/07 10:00	4/2/07 11:43	37.9	36.3	37.8	1x14h ptd
X756	62.5	4/5/07 02:00	4/2/07 11:12	37.8	36.0	37.5	1x3h ptd
X532	75	4/5/07 15:00	4/2/07 11:43	38.4	36.2	37.7	1x1h ptd
X538	71	4/5/07 11:00	4/2/07 12:10	39.0	37.3	38.8	1x4h ptd
X775	76	4/26/07 14:02	4/23/07 09:38	39.6	37.8	39.3	0
X784	58	4/25/07 20:02	4/23/07 10:18	38.1	36.2	37.7	1x1h
X789	80.5	4/26/07 19:20	4/23/07 10:57	40.0	37.7	39.2	1x6h ptd
X790	52.5	4/25/07 16:01	4/23/07 11:30	39.5	37.2	38.7	0
X774	51	4/25/07 15:02	4/23/07 12:02	38.8	37.3	38.8	1x1h

MO comment: A number of animals had an occasional short run (~1 hour long) of temperature below fever threshold after the onset of fever was documented. The difference between this study and those conducted at Battelle was that the animals were on continuous IV saline infusion that in the opinion of the reviewer can modify the natural course of the disease and allows the animals to stay hydrated despite their inability to feed. In addition the fluid infusion might have modified animal's febrile response.

Radiographic Changes

Chest and abdomen radiographs were taken at the time of anesthesia prior to aerosol exposure on Day 0 as baseline and to record the position of the femoral vein catheter. A second image was taken between exposure and necropsy, depending on the presence of increased respiration, as determined by the Investigator.

Pre-exposure chest radiographs were clear except for animal X666 where small right caudal opacity thought to represent bronchial calcification was noted. Approximately 72 hours

postexposure, single small infiltrates were evident in 6/9 animals. By Day 4 existing infiltrates enlarged and multiple opacities developed. There were no pleural effusions noted.

Table 29 Summary of Radiological Findings

Animal No.	Study Day		
	0	3	4
X532	Clear	Clear	Small left sup. Caudal
X538	Clear	Small infiltrate right proximal and caudal	Big right caudal left cranial
X666	Small right caudal shadow	Small right caudal shadow	Small right caudal shadow No infiltrate
X705	Clear	Infiltrate left middle	Natural Death? - whiteout
X756	Clear	Small left cranial	Big left cranial and middle
X774	Clear	Clear	Diffuse infiltrate every lobe
X775	Clear	Small infiltrate right middle	Large right middle and caudal
X784	Clear	Small right middle	Infiltrates in every lobe except right caudal
X789	Clear	Clear	Natural Death - whiteout (30 min post death)
X790	Clear	Natural Death - whiteout	ND

MO comment: Radiologic findings are consistent with progressive multifocal pneumonia. As a diagnostic tool CXR has minimal value due to rapid onset and fulminant course of the disease with a delay of radiographic evidence of pneumonia relative to symptom onset and death.

Laboratory Findings

Hematology

Complete blood count with differential was performed at baseline and on postexposure Day 1, Day 2, Day 3, and Day 4. Leukocytosis was observed in 6/10 animals immediately after aerosol exposure with *Y. pestis*. By Day 4 significant leukocytosis was observed (up to 66,420 cells/mm³) in 4/8 surviving animals. This was accompanied by an increase in absolute neutrophil count (ANC) from an average of 2,840 cells/mm³ on Day 1 to 15,710 cells/mm³ on Day 4. Leukopenia was observed in 2/8 surviving animals on Day 4.

As the disease progressed, hemoglobin/hematocrit levels and platelet count decreased relative to baseline, although the observed decreases were not statistically significant.

Table 30 Changes in Hematology Parameters

Cell Element	Day -7	Day 1	Day 2	Day 3	Day 4
WBC	10.68 (4.34)	9.60 (3.65)	10.56 (3.39)	17.50 (10.45)	23.65 (21.07)
Hemoglobin	14.80 (1.81)	15.04 (1.38)	14.55 (1.23)	14.16 (1.61)	13.44 (1.86)
Hematocrit	45.95 (4.66)	45.85 (3.37)	43.80 (2.97)	42.37 (4.80)	40.56 (5.28)
Abs. neutrophil count	4.12 (3.77)	2.84 (2.08)	2.39 (1.05)	10.62 (7.50)	15.71 (13.37)
Platelets	457 (115)	473 (107)	447 (107)	390 (143)	369 (183)

Adapted from study report

MO comment: Changes in hematology parameters are consistent with infectious process and inflammatory response.

Chemistry

Analysis of serum chemistry was performed at baseline (Day-7) and postexposure on Days 1, 2, 3, and 4. Abnormalities in serum chemistry were limited to LFTs. Levels of lactate dehydrogenase (LDH) became elevated following exposure as well as the levels of aspartate and alanine aminotransferases (AST and ALT). Serum blood urea nitrogen (BUN) and creatinine levels did not elevate and the Sponsor attributed this to the daily infusions of normal saline to maintain the animal's adequate circulating blood volume and to preserve renal function.

Table 31 Changes in Serum Chemistry

Assay	Study Day									
	-7		1		2		3		4	
NA-S (mmol/L)	147.4	(3.2)	148.5	(1.6)	148.4	(2.1)	145.2	(2.4)	145.1	(1.9)
K-S (mmol/L)	3.9	(0.6)	4.3	(0.6)	4.6	(0.8)	4.2	(0.9)	4.1	(0.8)
CL-S (mmol/L)	100.5	(2.8)	104.9	(1.9)	103.5	(2.1)	101.8	(3.2)	99.5	(5.0)
GLU (mg/dL)	214.1	(135.2)	204.5	(51.5)	185.9	(44.2)	127.7	(61.4)	100.9	(28.0)
BUN (mg/dL)	16.9	(4.6)	22.1	(6.8)	14.4	(3.7)	16.4	(10.9)	18.4	(13.8)
CRE-S (mg/dL)	0.7	(0.1)	0.8	(0.1)	0.7	(0.1)	0.7	(0.3)	0.9	(0.4)
PHOS (mg/dL)	6.3	(1.1)	6.9	(1.3)	5.3	(1.0)	5.3	(1.8)	4.6	(0.6)
BILI-T (mg/dL)	0.1	(0.1)	0.1	(0.1)	0.2	(0.1)	0.2	(0.1)	0.3	(0.1)
ALP (IU/L)	324.1	(144.7)	342.9	(135.8)	328.5	(124.7)	352.3	(114.2)	382.1	(135.3)
ALT (IU/L)	89.9	(45.9)	83.7	(53.6)	101.0	(44.0)	104.2	(38.1)	111.4	(50.4)
AST (IU/L)	41.5	(10.8)	53.0	(16.2)	104.9	(49.7)	166.2	(154.6)	139.8	(124.0)
GGT (IU/L)	34.6	(8.5)	36.0	(9.8)	38.0	(12.1)	49.7	(31.2)	63.4	(72.8)
TP (g/dL)	7.4	(0.4)	7.1	(0.5)	6.9	(0.5)	6.8	(0.5)	6.9	(0.6)
ALB (g/dL)	4.8	(0.3)	4.5	(0.4)	4.4	(0.3)	4.2	(0.4)	3.9	(0.5)
CA (mg/dL)	10.5	(0.6)	9.9	(0.3)	10.0	(0.5)	9.1	(1.1)	9.2	(0.5)
LDH (IU/L)	263.4	(60.8)	354.0	(45.4)	732.4	(329.2)	1206.1	(967.6)	2076.0	(2515.5)
GLOBN (g/dL)	2.6	(0.4)	2.6	(0.5)	2.5	(0.5)	2.7	(0.5)	3.1	(0.8)
A/G Ratio	1.9	(0.1)	1.7	(0.5)	1.8	(0.6)	1.6	(0.5)	1.4	(0.5)
BUN/CR Ratio	26.5	(8.0)	31.0	(14.9)	21.5	(4.8)	23.3	(11.5)	19.1	(5.5)

Adapted from study report

MO comment: Elevations of LFTs and LDH, as well as hypoalbuminemia became more profound as disease progressed. The reviewer agrees with the sponsor's assessment on the absence of changes in renal function and Na, Cl metabolism likely to be attributable to maintenance saline infusions.

Coagulation Parameters

Coagulation panel was assessed at baseline and on postexposure Days 1, 2, 3, and 4. Slight prolongation of the activated partial thromboplastin time (aPTT) was consistent with generalized inflammatory process.

Arterial Blood Gas

Arterial blood was obtained for blood gas examination 1 to 2 weeks pre-exposure, then daily from Day 1-5 post-exposure based on clinical observations, and at euthanasia. For all animals, ABG parameters, including oxygen saturation were consistently maintained within normal limits (or within baseline values) at most time points. Prior to euthanasia, however, a trend towards a decreasing and modestly depressed pO₂ was observed.

Blood Cultures

Venous blood was obtained for quantitative blood cultures. All animals were bacteremic with the *Y. pestis* CO92 strain. The first positive blood culture was detected between Day 2 to Day 4 post-exposure. Three animals became bacteremic on Day 2 while five were bacteremic on Day 3. The second positive blood culture a day after the initial positive blood culture was significantly greater in five out of seven animals.

Table 32 Quantitative Bacteremia (cfu/ml)

Group	Animal No.	Study Day					
		-7	0	1	2	3	4
2-Apr-07	X666	BLD	ND	BLD	BLD	1.9E+03	9.3E+02
	X705	BLD	ND	BLD	1.6E+01	1.4E+05	—
	X756	BLD	ND	BLD	BLD	BLD	1.7E+02
	X532	BLD	ND	BLD	BLD	5.5E+01	6.6E+02
	X538	BLD	ND	BLD	BLD	3.7E+02	2.1E+02
23-Apr-07	X774	BLD	ND	BLD	8.0E+02	1.2E+04	>2.0E+06
	X775	BLD	ND	BLD	BLD	BLD	5.4E+02
	X784	BLD	ND	BLD	BLD	6.7E+01	1.0E+03
	X789	BLD	ND	BLD	BLD	>2.0E+05	>2.0E+06
	X790	BLD	ND	BLD	1.0E+01	>2.0E+06	—

BLD = below detectable limit.

ND = not determined.

MO comment: *Bacteremia was observed in 100% of animals in this natural history study. However, due to infrequent sampling (once daily) correlation between bacteremia and fever onset is difficult to establish. As the majority of animals (7/10) were bacteremic prior to fever onset, it is likely that upon institution of more frequent sampling confirmation of bacteremic status of the animals prior to institution of therapeutic intervention could be ensured.*

Timing of death (TOD)

Respiratory rate recordings were used as the primary criteria for TOD determinations based on telemetry data. When respiratory rate values decreased and were no longer recorded, a “No Data” message was produced, which was an indicator death. If respiratory data were not available or did not clearly indicate a TOD, temperature was used as the secondary parameter. TOD obtained from temperature was determined when the temperature was < 36°C. Blood pressure recordings < 5 mmHg were also used to support TOD determinations. Telemetry blood pressure values are calculations equal to the average of systolic and diastolic blood pressures for each 30 sec time interval.

In this study animals survived to 72-100 h post challenge.

Table 33 Time to Onset of Fever ($\geq 1.5C$ above baseline for >2 hours) and Time to Death

Animal ID	Sex	Estimated inhaled dose (cfu)	Time to onset fever (h post-challenge)	Time to onset of bacteremia	Time from onset of fever to death (h)	Time to death (h post-challenge)
X666	F	7.21×10^4	67	71	29	96
X705	F	5.60×10^4	46	45	40	86
X756	M	8.94×10^4	62.5	92	32.5	95
X532	M	5.71×10^4	75	69	20	95
X538	M	5.85×10^4	71	68.5	22	93
X775	F	2.58×10^4	76	94	16	92
X784	M	3.19×10^4	58	69.5	42	100
X789	M	4.51×10^4	80.5	69	18.5	99
X790	M	1.54×10^4	52.5	45	20.5	73
X774	F	2.02×10^4	51	43	21	72
Mean \pm SD		$4.71 \times 10^4 \pm 2.39 \times 10^4$	64 \pm 11.8	66.6 \pm 18	26.2 \pm 9.2	90.1 \pm 10.0
Median		5.10×10^4	64.8	69	21.5	94

MO comment: Most animals succumbed to death within 33 hours of fever onset. Six animals in this study lived well under 24 hours after fever onset, reaffirming the need for early intervention in some animals. As in the previous study the blood for bacteremia was collected on the daily basis. The correlation between the fever onset and bacteremia therefore cannot be established. Nevertheless, six animals were bacteremic at the time of fever onset. There is a 100% correlation between onset of fever and subsequent death; all animals were febrile on the study postexposure and all succumbed to death.

Figure 4 Onset of Fever, Bacteremia, and Time to Death Post-Challenge

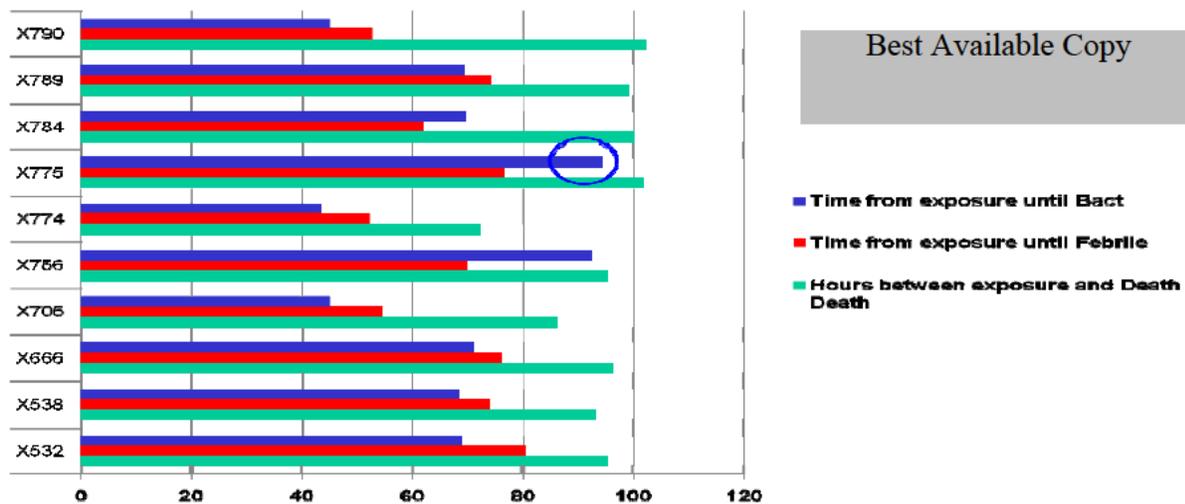
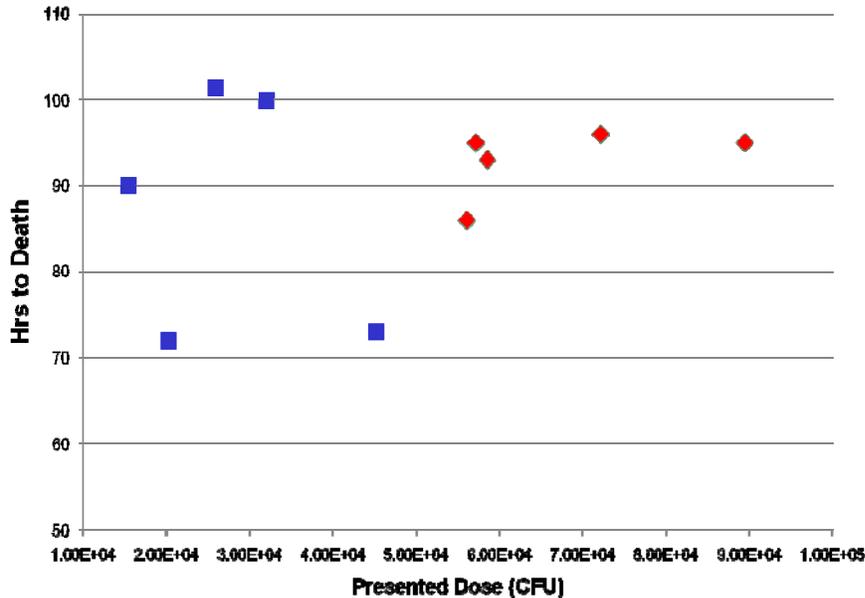


Figure 5 Onset of Death Relative to Challenge Dose



Best Available Copy

Adapted from the LRRRI presentation at the annual NIAID plague animal model update held on 02/24/2011

MO comment: *These graphical presentations of onset of fever, defined by the Sponsor as temperature $\geq 39^{\circ}\text{C}$ sustained for >2 hours, and death as well as the relationship between the challenge dose and onset of death (hours post challenge) were provided by LRRRI during NIAID plague animal model update on 02/24/2011. In the majority of animals (7/10) blood cultures became positive prior to sustained febrile response. The study design as it relates to infrequent daily blood sampling for culture might have played a confounding role in the absence of bacteremia prior to fever onset in 3/10 animals. There was no correlation between the challenge dose and the onset of death.*

Pathology

All animals in the study, those that were euthanized or succumbed to the disease, underwent necropsy.

All tissues were examined visually. Specific tissues were collected for histological examination (lung tissues with no lesions, lung tissues with lesions, liver, spleen, tracheobronchial lymph nodes, and brain) and quantitative bacteriology (lung tissues with no lesions, lung tissues with lesions, whole blood, liver, spleen, and tracheobronchial lymph nodes).

Gross and microscopic examination revealed that lungs were the primary organ for pathological findings, with consolidation, hemorrhage observed in 20-100% of the total lung tissue.

Histopathology findings were most notable for fibrinosuppurative patchy interstitial pneumonia. The tracheobronchial lymph nodes were affected as well, with the findings of disrupted nodal architecture and bacterial infiltration. Microscopic examination of the liver showed centrilobular necrosis with hypereosinophilia suggestive of hypoxic hepatic damage. In the spleen, congestion, neutrophilic leukocytosis, and basophilic rod bacteria consistent with *Y. pestis* were noted.

Table 34 Histopathology Findings

ID	X756	X532	X538	X775	X511	X666	X705	X774	X789	X790
Tissue/Sex	M	M	F	F	F	F	M	F	F	F
Death	Found dead	Found dead	Found dead	Found dead	Found dead	Found dead	Found dead	Found dead	Found dead	Found dead
LN bronchial	bacteria +++++, edema +++, leukocytosis +++++	bacteria +++++	bacteria +	bacteria +	bacteria ++, hemorrhage ++, leukocytosis +++++		bacteria ++, edema +++, hemorrhage ++, inflammation +++++	bacteria +++++, edema +++,	bacteria ++, edema +++++	bacteria +++++, edema +++++
Lung	bacteria +++++, hemorrhage +++++, inflammation +++++	bacteria +++++, hemorrhage +++++, inflammation +++++	bacteria +++++, hemorrhage +++++, inflammation +++++	bacteria ++, hemorrhage ++, inflammation +++++	bacteria +++++, hemorrhage +++++, inflammation +++++	bacteria +++++, hemorrhage +++++, inflammation +++++	bacteria +++++, hemorrhage +++++, inflammation +++++	bacteria +++++, hemorrhage +++++, inflammation +++++	bacteria ++, hemorrhage ++, inflammation +++++	bacteria +++++, hemorrhage +++++, inflammation +++++
Spleen	unremarkable	unremarkable	unremarkable	unremarkable	unremarkable	unremarkable	bacteria +++++	bacteria +++++	bacteria +++++	bacteria +++++
LN mandibular	bacteria +++++, hemorrhage+	bacteria +++++, hemorrhage++	unremarkable	unremarkable	unremarkable	bacteria +++++, hemorrhage+	bacteria +++++, hemorrhage++	unremarkable	unremarkable	unremarkable
Thoracic cavity		pleural fibrosis ++			pleural fibrosis +++++		pleural hemorrhage +++++		pleural fibrosis ++	pleural fibrosis +++++
Liver	hydropic changes ++	hydropic changes +++++	hydropic changes +	hydropic changes +++++	hydropic changes ++, congestion +	hydropic changes +++++		necrosis +++++	liver hydropic changes +++++	liver hydropic changes ++, congestion +
Stomach							multifocal hemorrhage ++			

MO comment: As in other natural history studies cardinal feature on the histopathology examination is fibrinosuppurative multifocal pneumonia, the finding consistent with the known pathological changes of pneumonic plague in humans.

Quantitative results from cultured organs are displayed in the table below.

Table 35 Quantitative Cultures, Select Tissues

Group	Animal No.	Study Day	CFU/g				
			Spleen	Liver	TBLN	Lung (L)	Lung (NL)
2-Apr-07	X666	4	1.2E+07	4.5E+06	4.3E+07	4.5E+09	1.10E+07
	X705	3	>1.5E+08	4.9E+08	8.0E+08	1.1E+09	1.80E+09
	X756	4	3.1E+06	1.5E+06	5.1E+05	7.5E+09	1.50E+08
	X532	4	3.2E+06	1.5E+06	7.9E+07	2.4E+09	8.30E+08
	X538	4	1.8E+05	1.6E+05	9.2E+06	1.8E+07	9.00E+06
23-Apr-07	X774	4	>1.4E+09	1.0E+07	2.9E+08	1.4E+09	2.50E+07
	X775	4	3.6E+06	2.0E+06	3.2E+08	6.3E+09	<5.8E+04
	X784	4	1.9E+06	7.7E+05	3.2E+08	2.7E+09	1.10E+08
	X789	4	>1.2E+09	>1.5E+08	2.3E+09	1.1E+09	7.00E+08
	X790	3	>1.3E+09	1.0E+09	3.3E+09	6.9E+08	6.20E+08

MO comment: *All target tissue cultured exhibited significant bacterial load.*

Conclusions:

The study results demonstrate that the inhalational model of pneumonic plague in AGM is uniformly fatal at exposures of >44 xLD50 (1.54×10^4). All animals developed an elevated temperature response (>1.5C over baseline sustained for >2 hours) to the infection approximately 84 hours post exposure and then succumb to the disease on average 26 hours later. High fever (>39C) was the most consistent sign of the disease in this study. Animals developed tachycardia and tachypnea at the time of temperature elevation. Laboratory abnormalities observed most often included neutrophilic leukocytosis, anemia, and elevation of liver enzymes. Coagulation abnormalities were also common. All animals were found to be bacteremic as early as Day 2 post challenge; in 6 of them positive blood culture preceded onset of fever (FDA definition). On necropsy/histopathology examination, changes in lungs, tracheobronchial lymph nodes were characteristic of pneumonic plague. The pathogen, route of exposure, clinical signs and symptoms, course of the disease as well as laboratory abnormalities and histopathology findings are fairly similar between AGM and humans with pneumonic plague. Selection of animals for treatment based on fever (trigger for intervention) is likely to result in a substantial number of animals bacteremic at the time of treatment administration and will allow for efficacy analyses to be conducted on a more conservative (microbiologically confirmed plague) animal population.

Study report “A Natural History Study of Pneumonic Plague in African Green Monkey”, U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) Study Protocol FY03-09G

The study was not Good Laboratory Practices (21 CFR Part 58) compliant. This study was conducted according to USAMRIID standard operating procedures (SOPs) and study-specific procedures (SSPs) where applicable. This study was conducted in USAMRIID’s Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facility.

***MO comment:** DSI inspections conducted on April 24-29, 2011 revealed that the study was of poor quality and lacked integrity, and the inspectors concluded that the study results were not reliable. Therefore, the reviewer will present summary data from the study for strictly documentation purposes. The findings from this study might only be included in the final cross natural study analysis and comparison to document consistency or inconsistencies in AGM plague model performance.*

Animals

Six AGMs (3 males and 3 females), weighing 3.5 to 6.0 kg, were selected from 10 trained AGMs with implanted telemetry devices to monitor temperature, pulse and respiratory rates. AGMs eligible for participation in the study must be experimentally naive and must pass a physical examination performed by a staff veterinarian within 30 days prior to assignment to the study. [CBC, full chemistry panel, and chest radiographs must all be normal at baseline.]

***MO comment:** No screen for prior exposure to environmental *Yersiniae* and *Y. pestis* was performed.*

Challenge Agent

The *Y. pestis* CO92 strain used in this study was originally isolated from a fatal human case of pneumonic plague (standard F1 positive CO92 strain used for plague studies at USAMRIID). The CO92 strain has antibiotic MICs comparable to other plagues strains collected from 1977 to 1998 by the California Microbial Diseases Laboratory. [REDACTED]

***MO comment:** For a detailed review of the LD50 study please refer to Clinical microbiology review by Dr. Simone Shurland for IND 64,429, SDN 055. The review recommends for efficacy studies in AGM to target a minimum exposure of 25LD50 to ensure the animals develop a lethal pneumonic infection.*

Study Procedures

- Anesthetized animals were exposed to a target dose of 100 ± 50 LD₅₀ [REDACTED] cfu) of *Y. pestis* in a head only dynamic aerosol chamber while respiratory minute volumes were measured by whole body plethysmography. Generated aerosol was sampled continuously. Aerosol concentrations of *Y. pestis* were calculated by plating out dilutions of the samples taken from impinger during the exposure.

- Animals were bled 7 to 14 days pre-exposure to obtain baseline values for CBCs. Then they were bled for blood culture, CBC, BUN, and Cr: at the time of exposure, daily post-exposure, at the time clinical signs onset and at the time of euthanasia/death.
- Monkeys were weighed daily.
- Clinical signs were documented at least twice daily after aerosol challenge.
- Temperature, BP, and heart rate were monitored every 30 minutes by telemetry. Vital signs were collected 7 days prior to exposure to obtain baseline values and to ensure devices were functioning. When fever developed (defined as $\geq 1.5^{\circ}$ C increase over baseline) in any of the monkeys, the AGMs were evaluated more frequently.
- Respiratory rates were obtained manually using a stopwatch.
- When clinical signs developed (i.e. fever and increase in respiratory rate defined as a 50% increase from baseline), animals were anesthetized, weighed, and bled for culture, CBC, BUN, and creatinine.
- Chest radiographs were taken prior to challenge, at the time when clinical signs developed, and at euthanasia or death.

Study Results

Exposure

While the target aerosol challenge dose was 100 ± 50 LD₅₀ () *Y. pestis*, the actual challenge dose for the AGMs was calculated to be between 9 and 57 LD₅₀. A single (first) animal received a dose within the target range of 100 ± 50 LD₅₀. The rest of the animals received progressively decreasing doses. The Investigators hypothesized that the reason for the below target exposure was inappropriate storage conditions of the aerosol stock (at room temperature) over the course of the challenge.

Table 36 Exposure Dose

Animal number	Inhaled dose of <i>Y. pestis</i>		Outcome
	LD ₅₀	cfu	
V627	57	1.9×10^4	Euthanized at 111.5 hr.
V514	30	1.0×10^4	Euthanized at 111 hr.
V569	23	8.0×10^3	Euthanized at 99.5 hr.
V113	21	7.2×10^3	Succumbed at 125 hr.
V605	9	3.1×10^3	Survived
V521	12	4.3×10^3	Survived

Clinical Observations

Temperatures were taken every 30 minutes via telemetry. Four AGMs exposed to >20 LD₅₀ of *Y. pestis* developed fever, defined as a temperature $\geq 1.5^{\circ}$ C increase over baseline, between 70 to 76 hours postexposure. Two animals exposed to <20 LD₅₀ neither developed a febrile response nor a change in their daily biorhythm.

A scoring system was used to monitor the animals postexposure to determine animal health status and the need for euthanasia. Table 37 below provides for the clinical parameters monitored and scoring for different levels of abnormal findings for each of them. A sum of the four individual parameter scores was recorded as a clinical score observed and provided below in Table 38.

Table 37 Clinical Observations Scoring

Activity	Behavior	Stimuli response	Breathing
Normal 5	Normal 5	Normal 5	Normal 5
Active 4	Antisocial 4	Enter room 4	Rapid 4
Slow Active 3	Depressed 3	Approach cage 3	Abdominal breathing 3
Sluggish 2	Hunched 2	Rattle cage 2	Dyspnea 2
Inactive 1	Ignoring 1	Pinch 1	Rales 1

Animals were to be euthanized if a total clinical observation score was ≤ 5 or animal was exhibiting any of the following conditions:

- Comatose, loss of consciousness
- Convulsions
- Abnormal respiration with rales on auscultation and/or radiographic findings of pulmonary fluid.

Table 38 Clinical Scores Observed on Study

Time (hrs)	V627	V514	V569	V113	V605	V521
24	20	20	20	20	20	20
48	20	15	20	20	20	20
72	20	16	20	16	20	20
76	20	16	20	16	20	20
80	19	10	20	16	19	20
83	17	8	18	13	ND	ND
96	20	11	17	14	20	20
99	15	10	12	13	20	17
103	14	11		12	20	19
107	11	8		12	20	20
111	9	5		11	20	20
116				11	20	20
119				13		
122				8		
125				5		

Respiratory rates were monitored manually at least twice daily along with clinical assessments. Increase in respiratory rate was defined as a 50% increase over baseline. The same four febrile animals were noted to have an increased respiratory rate over time. The report, however, did not specify the timing of onset of increased respiratory rate. The two surviving animals did not develop tachypnea.

Table 39 Respiratory Rates Postexposure

Time (hrs)	V627	V514	V569	V113	V605	V521
24	40	32	36	32	40	28
48	40	32	32	28	32	28
72	36	32	40	44	32	28
76	24	44	40	36	36	32
80	40	45	42	36	36	27
83	51	ND	42	32	ND	ND
96	36	52	100	60	36	32
99	80	88	128	72	36	32
103	104	120		108	32	32
107	123	140		118	36	32
111	140	146		124	36	34
116				154	26	28
119				162		
122				168		
125				168		

Four of the 6 AGMs showed clinical signs of the disease and subsequently succumbed to pneumonic plague. The remaining two animals, challenged with 9 LD₅₀ (3,100 cfu) and 12 LD₅₀ (4300 cfu) did not show any signs of clinical illness. Three of the AGMs were euthanized between 99.5 to 111.5 hrs after exposure (4.1 to 4.6 days after exposure) while one AGM succumbed to the disease 125 hours (5.2 days after exposure).

Laboratory Findings

CBC was performed at baseline and daily post-exposure. All AGMs had white blood cell counts within the normal range prior to inhalation exposure. At 24-72 hours post-exposure, leukocytosis developed in the four febrile animals. By Day 3 post-exposure, there is a lymphocyte/granulocyte inversion in the 4 animals that eventually succumbed to the disease. Monocyte percentages were constant throughout the study. The two surviving AGMs did not manifest any CBC abnormalities.

Table 40 WBC Count, Febrile Animals

Time (hr)	WBC Count				
	V627	V514	V569	V113	Mean
Baseline	8.6	6.33	5.6	7.8	7.08
24	9.7	10.1	12.5	6.6	9.72
48	12.6	10.5	11.6	9.9	11.15
72	16.9	19.8	16.9	15.3	17.22
83	11.4	13.3	12.0	13.3	12.5
96	23.7	11.1	41.0	12.8	22.15

The normal range of blood urea nitrogen (BUN) and creatinine for AGMS were determined to be 8-20 mg/dL and 0.5-1.1 mg/dL, respectively. Only AGM V569 had mildly elevated BUN levels at 83 hours (when clinical signs developed) and 96 hours post-exposure and mildly elevated creatinine levels throughout the study. AGM V605 and AGM V521 had mild elevations of creatinine at 48 hours and at 72 hours post-exposure, respectively.

Bacteremia

Four AGMs with fever and clinical signs developed bacteremia. AGM V113 had a positive blood culture at 48 hours post-exposure and AGM V627, V514, and V569 were bacteremic at 72 hours. V514 developed clinical signs at 80 hour post exposure and V627, V569, and V113 at 83 hours post-exposure. Bacteremia was persistent for these four AGMs until death. The two AGMs that did not develop fever did not have bacteremia.

Table 41 Bacteremia

Animal ID	Challenge Dose (LD ₅₀)	Time (h)						Terminal quantitative blood culture (cfu/mL)
		24	48	72	80	83	96	
V627	57	-	-	+	ND	+	+	8x10 ⁷ at 111.5 hr
V514	30	-	-	+	+	ND	+	3x10 ⁶ at 111 hr
V569	23	-	-	+	ND	+	+	9x10 ⁸ at 99.5 hr
V113	21	-	+	+	ND	+	+	1x10 ⁸ at 125 hr
V605	9	-	-	-	ND	ND	-	
V521	12	-	-	-	ND	ND	-	

Radiographs

Chest radiographs were taken prior to exposure, at the time when clinical signs were confirmed, and at euthanasia or death. The summary report provided chest radiograph images of each animal at each time point. The AGMs that succumbed to the disease showed evidence of pulmonary involvement at the time when clinical signs developed in the AGMs, which was around 80-83 hours post-exposure. None of the AGMs that survived had any chest radiographic changes.

Outcome

Out of six exposed animals four developed sustained elevation of body temperature, tachypnea and tachycardia over baseline and succumbed to death. Two animals exposed to the lowest challenge dose of *Y. pestis* aerosol (<20 LD₅₀ or 6.8 x 10³ cfu) survived. During the course of the experiment these survivors have not developed any signs of the disease, nor have they found to be bacteremic. Survivors were not euthanized and were not examined for the presence of subclinical evidence of infection.

Table 42 Timing of Fever, Bacteremia, and Onset of Death/Euthanasia

Animal ID	Challenge dose (LD ₅₀)	Challenge dose (cfu/mL)	Time to bacteremia (h)	Time to fever onset (h) (estimated)	Time to onset of clinical signs (h)	Time to death/euthanasia (h)	Time between fever onset to death (h)
V627	57	1.9 x 10 ⁴	72	76	83	111.5	35.5
V514	30	1.0 x 10 ⁴	72	73	80	111	38
V569	23	8.0 x 10 ³	72	70	83	99.5	29.5
V113	21	7.2 x 10 ³	48	74	83	125	51
V605	9	3.1 x 10 ³	None	Neg	Neg	--	--
V521	12	4.3 x 10 ³	None	Neg	Neg	--	--
Mean for 4 affected animals			60	73.25	82.25	111.75	38.5

Adapted from the study report

MO comment: Method for calculating baseline temperature has not been defined in the protocol. In addition, telemetry dataset does not contain information on the temperature recordings prior to exposure, thus precluding reviewer from verifying sponsor's estimated onset

of fever and from applying FDA fever definition to the animals in the study as it involves calculation of baseline average temperatures per hour.

Pathology

Necropsies and pathological examinations were performed on the four AGMs that succumbed to death after inhalational exposure to *Y. pestis*.

Table 43 Histopathological Findings, Lesion Severity

Animal Tattoo No.	Lungs	Mediastinal Lymph Nodes	Bronchial Lymph Nodes	Larynx and/or Trachea	Mediastinal Connective Tissue
V627	**	**	**	Neg	Neg
V514	***	**	**	**	Neg
V569	***	***	*	**	Neg
V113	***	**	**	Neg	**

*** Marked or Severe ** Moderate * Mild Neg. No Lesions

All AGMs developed lesions in the respiratory system and adjacent thoracic organs. These lesions consist of:

1. Pulmonary edema
2. Red or violaceous pulmonary discoloration
3. Tracheal or bronchial fluid
4. Mediastinal edema
5. Pleural effusion (except AGM V514)
6. Tracheobronchial lymphadenopathy.

Microscopically, the lesions consisted of edema, acute hemorrhage, and acute inflammation, alone or in combination. These changes were always associated with the presence of extracellular bacillary bacteria or intracellular bacteria in phagocytic inflammatory cells. The bacteria were morphologically compatible with *Y. pestis*. In all four monkeys, the lungs, bronchial lymph nodes, and mediastinal lymph nodes were affected to some degree, with the lungs affected most severely. Pathologic lesions developed in the larynx and trachea of two monkeys and in the mediastinal connective tissues of the other two monkeys.

Bacteria were seen in the spleens of all four animals died from the disease and mild to moderate inflammation was found in three. Bacteria compatible with *Y. pestis* was also noted in blood vessels of other organs in all four deceased animals. On necropsy microscopic fibrin thrombi in hepatic sinusoids with associated bacteria and hepatocellular degeneration with necrosis in association with *Y. pestis* were seen in one animal each.

Conclusions

The study results although deemed of poor quality by the FDA DSI inspectors demonstrate that the inhalational model of pneumonic plague in AGM is fatal at exposures of as low as 21 xLD₅₀ (7.2 x 10³ cfu) *Y. pestis* via aerosol. Doses of 9 and 12 x LD₅₀ did not result in fatal outcome, or in animals exhibiting any signs of illness, or blood culture positive for *Y. pestis* at any time postexposure. All animals exposed to the >20 xLD₅₀ dose developed an elevated temperature

response ($>1.5^{\circ}\text{C}$ over baseline sustained for >2 hours as stated in the study report) to the infection approximately 84 hours post exposure and then succumbed to the disease on average 38.5 hours later. High fever ($>39^{\circ}\text{C}$) was the most consistent sign of the disease in this study. Animals developed tachypnea at the time of temperature elevation. Laboratory abnormalities observed most often included neutrophilic leukocytosis. All febrile animals were found to be bacteremic. Bacteremia was detected as early as Day 2 post challenge; in 3/4 animals that developed fever positive blood culture preceded fever onset. On necropsy/histopathology examination, changes in lungs, tracheobronchial lymph nodes were characteristic of pneumonic plague. The pathogen, route of exposure, clinical signs and symptoms, course of the disease as well as laboratory abnormalities and histopathology findings are fairly similar between AGM and humans with pneumonic plague. Targeting aerosol exposures at $100\pm 50 \text{ xLD}_{50}$ will likely result in a symptomatic and uniformly fatal disease. Selection of animals for treatment based on fever (trigger for intervention) is likely to result in a substantial number of animals bacteremic at the time of treatment administration and will allow for efficacy analyses to be conducted on a more conservative (symptomatic microbiologically confirmed plague) animal population.

Discussion

Human primary pneumonic plague is extremely uncommon in the US and the interventions to treat it cannot be studied in randomized controlled trials. It is a severe disease characterized by relatively short incubation period of 1 to 6 days after a close contact with person or animal with high bacterial burden in respiratory secretions (usually secondary pneumonic plague cases). Infectious inoculum of *Y. pestis* in humans is small and is estimated at 100-500 cfu delivered via droplet aerosol. Disease manifests by sudden onset of chills, high fever, and chest pain followed by nonproductive cough and dyspnea. Primary pneumonic plague has a fulminant course and if left untreated progresses to hemoptysis, ARDS, shock, multiorgan failure and death within 2 to 6 days. The most prominent feature on autopsy of persons who succumbed to pneumonic plague is fibrinosuppurative bronchopneumonia. Streptomycin and tetracyclines are approved for the treatment of plague in the US. Although not approved, gentamicin has been used successfully in some cases published.

The review of published literature was conducted to identify cases of primary pneumonic plague with sufficient characterization of the course of the disease to enable comparison with the animal model. Table 44 below provides a summary of the cases reviewed.

Table 44 Human Cases of Primary Pneumonic Plague

Cases primary pneumonic plague	Incubation period	Symptoms	Time to death	Diagnostics	Pathology
39 cases of primary pneumonic plague Mukden 1946 (JID Vol. 82, No. 1 pp 52-58)	4 days (2-7)	fever, max at 24 h, headache, chest pain, cough on day 2, hemoptysis day 2 -3, malaise, rales on auscultation	3 days (2-6) 3/5 treated with sulfadiazine survived (2 treated on day 1 of symptoms, others on day 3 or later)	Microscopy and culture of the sputum, guinea pig inoculation	Not described
47 yo F primary pneumonic plague from sick cat exposure (JAMA 1984;251:929-31)	3 days	Chills, myalgia, chest pain, cyanosis, N/V, neutropenic, DIC, rales, CXR lobar pneumonitis	3 days	Sputum smear – GNRs, postmortem culture of sputum and lung + <i>Y. pestis</i>	Fibrinosuppurative pneumonia
2 primary pneumonic plague cases in Uganda 2004 (Emerg Infect Dis. 2006 Mar;12(3):460-7)	5 days	Headache, fever, chills, chest pain, dyspnea, weakness, cough, pneumonia on auscultation	3 days 1 survivor with chloramphenicol	PCR, culture (CO92?), fluorescent microscopy; immunochromatographic assay	Not described
31 yo M primary pneumonic plague cat transmitted (Am. J. Trop. Med. Hvg., 51(1), 1994, pp. 109-114)	3 days	Abdominal pain, fever, nausea, diarrhea, cough. R lobar pneumonia	4 days	Sputum microscopy GNRs; culture + postmortem	RUL consolidation, patchy fibrinosuppurative bronchopneumonia, splenomegaly
37 yo M primary pneumonic plague from mountain lion (CID 2009 Aug 1;49(3):e33-8)	3-3.5 days	Fever, chills, nausea, vomiting, myalgia, cough, hemoptysis, mild hypoxia	5-6 days	Postmortem culture positive from multiple organs including brain	Hemorrhagic pneumonia R lung.
Case 18 cat associated plague (31 yo F) (CID 2000 Jun;30(6):893-900)	4 days	Vomiting, fever, myalgia, malaise, neutrophilic leukocytosis, hypoxia, shock,	Survived treated with on day 2 of symptoms	Bronchoscopic washings and sputum + fluorescent antibody assay	n/a
12 cases of primary pneumonic plague dog associated with subsequent human-human transmission (CID 2011 52 (2): 185-190)	2 days (1-5)	Fever, chills, cough, hemoptysis, chest pain, vomiting, fatigue, dyspnea	3/12 died (1, 6, 7 days after symptom onset despite treatment with antibiotics and supportive care)	Culture (<i>Y. pestis</i> biovar <i>antiqua</i>) or serology	Not described
Case 19 cat associated plague (15 F) (CID 2000 Jun;30(6):893-900)	6 days	Headache, fever, delirium, respiratory distress, non productive cough, hypoxic, RUL consolidation, necrosis on CXR	Survived treated on day 4 of symptoms	Blood culture from the patient? and infected cat became + 7 days after admission	n/a

African Green Monkey (AGM) model of primary pneumonic plague has been evaluated in 4 natural history studies conducted by 3 independent laboratories and reviewed by DAIP. This animal model is proposed to be used as an animal model for evaluating efficacy of products for treatment of pneumonic plague under the Animal Rule, 21 CFR 314 Subpart I.

Table 17 below summarizes findings in the natural history studies of primary pneumonic plague in AGM.

Table 45 Summary Findings, AGM Primary Pneumonic Plague Natural History Studies

Parameter	Battelle Study 617-G607610	Battelle Study 875-G607610	LLRI	USAMRIID
Average <i>Y. pestis</i> Exposure Dose of (range)	614 LD ₅₀ or 2.09x10 ⁴ cfu (106 LD ₅₀ to 1150 LD ₅₀) (3.59 x10 ⁴ to 3.91 x10 ⁵)	48 LD ₅₀ or 1.64x10 ⁴ cfu (24 LD ₅₀ to 88 LD ₅₀) (8.24 x10 ³ to 2.98 x10 ⁴)	134 LD ₅₀ or 4.71x10 ⁴ cfu (44 LD ₅₀ to 255 LD ₅₀) (1.5 x 10 ⁴ to 5.6 x 10 ⁴)	9 LD ₅₀ to 57 LD ₅₀ (3.1 x 10 ³ to 1.9 x 10 ⁴)
Animals	3 males and 7 females	5 males and 5 females	5 males and 5 females	3 males and 3 females
Mortality	100% 10 found dead	100% 10 found dead	100% (3 found dead, 7 euthanized)	67% (4/6: 1 found dead, 3 euthanized)
Average Time to Death (range)	72 hours (61 to 90 hours)	93.5 hours (67.5 to 139 hours)	90 hours (72 to 100 hours)	112 hours (99.5 to 125 hours)
Average Time to Fever Onset (range)	44 hours (39 to 49.5 hours)	54 hours (47 to 61.5 hours)	64 hours (46 to 80.5 hours)	73 hours (70 to 76 hours)
Average Interval between Fever and Death (range)	28.5 hours (16 to 44.5 hours)	39 hours (17.5 to 78 hours)	26 hours (16 to 42 hours)	38.5 hours (29.5 to 51 hours)
Initial Level of Bacteremia (cfu)	33 to >650,000 cfu/mL	600 cfu to >7,000,000 cfu/mL	10 cfu to >200,000 cfu/mL	3 x 10 ⁶ to 9 x 10 ⁸ cfu/mL (terminal)
Timing of Bacteremia Onset (range)	48 to 72 hours (100% + by Day 2)	43.5 hours (30 to 68.5 hours)	66.5 hours (43 to 94 hours)	60 hours (48 to 72 hours)
Radiographic abnormalities	n/a	n/a	5/10 on Day 3	4/4 at 80 - 83h postexposure
Symptoms	Fever, loss of appetite, respiratory distress, bloody respiratory secretions, lethargy	Fever, hunched posture, respiratory distress, bloody respiratory secretions, lethargy	Fever, loss of appetite, diarrhea, lethargy	Fever, loss of appetite, respiratory distress, lethargy
Pathology	hemorrhagic pneumonia	hemorrhagic pneumonia	fibrinosuppurative hemorrhagic pneumonia	fibrinosuppurative hemorrhagic pneumonia

Review of the above studies and comparison of critical elements of AGM model to human primary pneumonic plague cases available for review in public domain revealed close similarity between the animal and human disease. The AGM model performed consistently in 3 different laboratories with minor variations as presented in Table 45.

The challenge agent used in all four studies was *Y. pestis*, biovar *orientalis*, strain CO92, recovered from a person with pneumonic plague³. This biovar is common in isolates from humans with plague during the last century and remains a relevant human pathogen.⁴

Representative **virulence factors**, including capsular F1, secreted protein V, and plasminogen activator, are highly conserved between 3 known biovars of *Y. pestis* and play significant role in the disease pathogenesis and progression in both humans and animals.⁵

Close contact with an infected human or animal in the last phase of the disease, characterized by the production of highly infectious hemorrhagic sputum has been simulated in AGM model by

³ Doll, J. M., P. S. Zeitz, P. Ettestad, A. L. Bucholtz, T. Davis, and K. Gage. 1994. Cat-transmitted fatal pneumonic plague in a person who traveled from Colorado to Arizona. *Am. J. Trop. Med. Hyg.* 51:109-114

⁴ Drancourt M, Roux V, Dang LV, Tran-Hung L, Castex D, Chenal-Francisque V, et al. Genotyping, *Orientalis*-like *Yersinia pestis*, and plague pandemics *Emerg Infect Dis.* 2004 Sep.

⁵ Prentice MB, Rahalison L. Plague. *Lancet.* Apr 7 2007;369(9568):1196-207

head only *Y. pestis* aerosol exposure. In addition, inhalational route of exposure is most likely to be used during a potential bioterrorism event involving *Y. pestis*.

The target aerosol exposure of 3.43×10^4 cfu is approximately 60-300 times greater than the estimated infectious inoculum in humans of 100-500 cfu⁶ and is estimated to represent 100 x LD₅₀ for AGM. The data for determination of LD₅₀ in AGM was not reviewed by DAIP; however, upon evaluation of the data from 4 AGM natural history studies the review team noted that despite significant variability in the actual challenge dose received (9 to >1000 x LD₅₀), exposures above 7.2×10^3 cfu (corresponding to about 20 x LD₅₀) induce signs and symptoms of pneumonic plague and are uniformly fatal. In outbreak investigations the persons in close contact with the index case in the late phase of their disease were the most susceptible. Airborne droplet transmission is hypothesized for this form of the disease in humans. *Y. pestis* could easily be weaponized with infectious aerosols delivered over large densely populated geographic areas resulting in high exposure/disease rates.⁷

The incubation period for primary pneumonic plague on average is 3 days and is similar between humans and AGM as is the duration of illness prior to death again averaging about 3 days (see Tables 44 and 45 above). Calculation and comparison of the time course of the disease post exposure is feasible due to the characteristic abrupt onset of **signs/symptoms** – chills and fever in humans and fever in AGM. It is worth noting that the temperature in AGM rises sharply and invariably persists until the time of death. Malaise and chest pain reported in humans cannot be readily assessed in the animals due to inability to verbalize; however, cough, tachycardia and tachypnea, direct signs of CV and respiratory organ system involvement, are manifested by both species as the disease progresses. Extensive parenchymal inflammation of the lung tissue and CV collapse with coagulation abnormalities lead to death in men and AGM with primary pneumonic plague. Neutrophilic leukocytosis, metabolic acidosis, elevation of liver enzymes and creatinine as well as prolonged PT/PTT were observed in some animals late in the disease and correspond to metabolic, hematologic and coagulation abnormalities seen in humans with primary pneumonic plague.

Due to the sporadic nature of the disease in man, particularly in the US, **diagnosis of plague** in an index case requires high index of suspicion with meticulous collection of the exposure history and in many cases is established postmortem due to the fulminant nature of the disease (median time to death 3 days) and the limitations of bacteriological identification (3-4 days for culture results). In cases with survival outcome, the diagnosis was either suspected early during outbreak investigation and/or patients were treated empirically shortly after disease onset with broad spectrum antibiotics for pneumonia allowing patients to survive to modification of therapy after pathogen isolation and identification. In the AGM model, the **therapeutic window for intervention**, i.e. the time from onset of disease as manifested by fever (definitions varied between the natural history studies from 39°C to >1.5°C elevation over the baseline for 2 consecutive hours) to death is on average 2 days, while in humans this average is 1 day longer. In addition, in some animals (30-50% depending on the study) death occurred in less than 24 hours after onset of fever unrelated to the challenge dose. All animals that developed fever after challenge were bacteremic on day 2-4 of illness and also at the time of death. As microbiology

⁶ Franz DR, Jahrling PB, Friedlander AM, et al. Clinical recognition and management of patients exposed to biological warfare agents. JAMA. 1997;278:399-411

⁷ Inglesby TV et al. Plague as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. JAMA. 2000 May 3;283(17):2281-90.

evaluations in submitted natural history studies were done infrequently (once daily), correlation between onset of fever and onset of bacteremia cannot be assessed precisely. However, up to 50% of animals were found to be bacteremic prior to onset of fever and quantitative blood cultures exhibit exponential increase in bacterial counts over time, suggesting that animals that were not bacteremic prior to onset of fever are likely to be positive at the time of fever onset. Real time confirmation of bacteremia prior to treatment is not feasible due to delay in culture results. Thus, it is reasonable to suggest that the fever defined as $>1.5^{\circ}\text{C}$ for 2 consecutive hours could be used as a **trigger for intervention** in this animal model, provided that the animals evaluable for efficacy have blood collected for culture before or at the time of treatment initiation, and subsequently demonstrate bacterial growth of *Y. pestis*. Differences between the studies in the definition of fever could be considered minor as the timing of the onset of fever as defined by individual studies differed from one another by 1 hour or less if applied to the same temperature graph. Therapeutic intervention is to occur some time after trigger positivity. Preliminary results from treatment trials conducted by NIAID show that initiation of treatment was typically within 6 hours after fever. The sponsor cites feasibility concerns related to the complicated dosing schedule of antimicrobials to be studied in an attempt to match human exposures at the approved doses. Although treatment initiation shortly after trigger positivity is a concern expressed by some in DAIP, the reviewer is confident that a conservative approach to the **case definition of established pneumonic plague** in an AGM exposed to *Y. pestis* aerosol as evident by the presence of fever and positive blood culture collected prior to therapeutic intervention will allow for the selection of an animal population with disease comparable to that of a subgroup of patients with primary pneumonic plague seeking medical attention after the onset of symptoms. It will also allow for greater sensitivity in evaluation of the **treatment effect of an intervention**, a statistically significant improvement in Day 28 infection free survival as compared to placebo. Relatively short duration of illness in AGM and in humans alike argues against a delay in the institution of treatment after manifestation of the disease in AGM. Such delay was demonstrated to result in fatal outcome in humans (published cases, see table above) and is likely to significantly reduce sensitivity in demonstrating therapeutic effect of an intervention in animals when a significant number of animals do not survive to treatment or the intervention is administered shortly before death. Delay beyond 12 hours after onset of fever would require larger, more labor intensive trials in BSL-3 confinement with unnecessary waste of study animals.

The statistical team has provided DAIP with sample size calculations to achieve at least 80% of power on the assumptions that some animals will not exhibit signs of the disease postexposure and some will not be bacteremic at the time of therapeutic intervention. Statistically significant results are expected to be achieved if the difference between the study and the placebo arms is at least 7 animals, assuming equal sample size per arm of 9 and above, and as low as 6, assuming equal sample size of 7-8 animals/arm.

Table 46 Essential Data Elements of an Animal Model

DATA ELEMENTS	Animal(s)	Human
A. Characteristics of the CBRN Agent that Influence the Disease or Condition		
1. The challenge agent	<i>Y. pestis</i> CO92 strain at proposed inhalational dose of 3.49×10^4 cfu (100±50 x LD ₅₀)	<i>Y. pestis</i> (CO92 strain was isolated from a human with pneumonic plague)
2. Pathogenic determinants	F1 capsular antigen, V antigens and Yersinia outer membrane proteins (yops)	

DATA ELEMENTS	Animal(s)	Human
	antigens, plasminogen activator (Pla)	
3. Route of exposure	Aerosol (head only exposure)	Aerosol (direct aerosol exposure to a close contact with pneumonic plague or bioweaponized aerosol)
4. Quantification of exposure	3.43×10^4 cfu ($100 \pm 50 \times LD_{50}$)	Infectious inoculum 100-500 organisms. Variable level during close aerosol contact, high level possible if bioweaponized
B. Host Susceptibility and Response to Etiologic Agent	highly susceptible	highly susceptible
C. Natural History of Disease: Pathophysiologic Comparability		
1. Time to onset of disease/condition	26-80 hours (1-3) days to fever onset	1-6 days to fever and other symptom onset
2. Time course of progression of disease/condition	Time to death 15-75 hours (1-3 days) (median 2 days)	With no antibiotics survival 2-6 days (median 3)
3. Manifestations (signs and symptoms)	Fever, hunched posture, inappetence, lethargy, tachypnea, tachycardia, cough, bloody respiratory secretions, neutrophilic leukocytosis, coagulation abnormalities	Abrupt onset of fever, chills, lethargy, headache, myalgias, arthralgias, vomiting, diarrhea, cough, hemoptysis, followed by respiratory distress, ARDS, DIC, multiorgan failure, shock, neutrophilic leukocytosis, thrombocytopenia, acidosis CXR: multilobar consolidation, cavities or bronchopneumonia
4. Pathology	Hemorrhagic pneumonia	Hemorrhagic pneumonia
D. Trigger for Intervention	Fever (>1.5 C over baseline sustained for 2 hours)	Variable depends on high index of suspicion, positive history, hemorrhagic pneumonia; positive smear or culture, titers of anti-F1 ab
E. Characterization of the Medical Intervention		
1. Product class	fluoroquinolones	
2. Mechanism of action	Inhibition of DNA gyrase and topoisomerase IV	
3. In vitro activity	Active against <i>Y. pestis</i>	
4. Activity in disease/condition of similar pathophysiology	Not yet studied	Approved for the treatment of community acquired pneumonia and hospital acquired pneumonia.
5. PK in unaffected animals/humans	Proposed dosing regimen is humanized to achieve exposures observed in humans at 500 mg dose	Described in levofloxacin PI
6. PK/PD in affected animals/humans	Proposed dosing regimen is humanized to achieve exposures observed in humans at 500 mg dose	Described in levofloxacin PI
7. PK interactions with medical products likely to be used concomitantly	Not applicable	Not applicable

DATA ELEMENTS	Animal(s)	Human
8. Synergy or antagonism of medical products likely to be used in combination	Not applicable	Not applicable
F. Design Considerations for Animal Efficacy Studies		
1. Endpoints	mortality	mortality
2. Timing of intervention	after fever onset	Variable upon suspicion, time of diagnosis, or general condition -- empiric
3. Route of administration	IV	IV
4. Dosing regimen	Humanized	Approved for serious infections

The AGM model of pneumonic plague in the opinion of the reviewer is sufficiently well characterized for its susceptibility to a human pathogen (*Y. pestis CO92*) and pathophysiology of the disease (comparable route of exposure, incubation period, manifestation, duration, and outcome of the disease). The correlation between the proposed trigger for intervention (fever onset) and the onset of bacteremia was not well established in the studies reviewed due to infrequent blood sampling for culture and will need confirmation during efficacy studies (the blood for culture needs to be collected at least at the time of trigger positivity, or immediately prior to treatment administration). Infection free survival should be an outcome measure for the efficacy study in pneumonic plague.

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/s/

YULIYA I YASINSKAYA
04/20/2012

JOHN J ALEXANDER
04/20/2012

CLINICAL REVIEW

Application Type	sNDA
Application Number(s)	NDA 20-634;21-721; 20-635
Priority or Standard	Priority
Submit Date(s)	October 27, 2011
Received Date(s)	October 28, 2011
PDUFA Goal Date	April 28, 2012
Division / Office	DAIP
Reviewer Name(s)	Elizabeth O'Shaughnessy, M.D.
Clinical Team Leader	John Alexander, M.D., M.P.H.
Review Completion Date	March 26, 2012
Established Name	Levofloxacin
(Proposed) Trade Name	Levaquin [®]
Therapeutic Class	Antibacterial
Applicant	Janssen Pharmaceuticals (Johnson & Johnson)
Formulation(s)	IV solution
Dosing Regimen	
Indication(s)	Pneumonic Plague
Intended Population(s)	Adults, Pediatric age group \geq 6 months

Template Version: [March 6, 2009](#)

Submissions related to sNDA:

NDA 20634/S-061, SN 72, 76, 78, 80, 81, 85, 87, 88, 89; SDN 1244, 1248, 1250, 1253, 1255, 1259, 1262, 1263, 1264 (10/27/2011, 11/23/2011, 12/21/2011, 12/23/2011, 1/12/2012, 2/23/2012, 3/5/2012, 3/12/2012, 3/30/2012);

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1 Recommendations/Risk Benefit Assessment

1.1 Recommendation on Regulatory Action

The clinical reviewer recommends approval of levofloxacin for the treatment of human pneumonic plague. The supplemental NDA has met the following criteria for approval of a drug under the “*New Drug and Biological Drug Products; Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible*,”¹ 21 CFR 314.600-650 for New Drugs, also referred to as “*The Animal Rule*”.

Criteria:

- Pathophysiology of the disease and product’s mechanism of action are reasonably well understood;
The pathophysiology of pneumonic plague is well understood, humans subjects develop a fibrino-suppurative hemorrhagic pneumonia with or without septicemia which is similar to the pathophysiology of pneumonic plague in the African Green monkey model. The mechanism of action of levofloxacin is also well understood. Levofloxacin belongs to the fluoroquinolone class of antibacterial agents. Levofloxacin and others in the fluoroquinolone class directly inhibit DNA synthesis through the interaction of the drug complexes with one or both of the bacterial type II topoisomerase enzymes, DNA gyrase and topoisomerase IV. The type II topoisomerases play essential roles in bacterial DNA replication, transcription, repair, and recombination.
- The product efficacy is demonstrated in more than one animal model, unless animal model is sufficiently well characterized for predicting the response in humans;
The African Green monkey (AGM) model of pneumonic plague mimics the human disease in most clinical and pathological characteristics of the disease. The AGM therefore, provides a suitable model to evaluate the efficacy of antibacterial treatment for pneumonic plague, a bioterrorism-associated disease which is not feasible to study in humans for ethical reasons.
Post-exposure treatment with IV levofloxacin for established pneumonic plague was demonstrated to be efficacious in preventing death in African Green monkeys infected with lethal doses of aerosolized *Yersinia pestis*. The efficacy of the levofloxacin was demonstrated in an A G M model of pneumonic plague in which treatment with a humanized dose of levofloxacin achieved a 94% (16/17) survival rate.
- The animal study endpoint of survival is clearly related to the desired benefit in humans.

¹ Final Rule published in the Federal Register, Vol. 67, No. 105, May 31, 2002, pages 37988-37998; Regulations: 21 CFR § 314.600-650 (New Drugs)

Untreated pneumonic plague is a fatal disease. The primary endpoint in the efficacy study of survival is, therefore a relevant endpoint in human patients with pneumonic plague. Information on the pharmacokinetics and pharmacodynamics of levofloxacin in animals and humans allowed selection of an efficacious dose in AGM that matched a known effective human dose of 500mg daily.

Levofloxacin, approved by the FDA in 1996, is a widely prescribed antibiotic for multiple indications including nosocomial and community acquired bacterial pneumonia and inhalational anthrax, post exposure. Pre-market safety was assessed in 7,537 patients in 29 pooled Phase 3 clinical trials. An estimated (b)(4) treatment courses were dispensed in 2010 per the applicant's database. The drug label has been updated to include post-market safety information and the most recent updated labeling for LEVAQUIN® was approved by the Agency on 09 June 2011.

The reviewer finds that the safety information outlined in the current levofloxacin label provides sufficient to support the approval of Levofloxacin for the proposed indication of pneumonic plague.

1.2 Risk Benefit Assessment

Pneumonic plague is a highly lethal condition. Aerosolized *Y. pestis* caused a rapidly progressive bacterial pneumonia and death in untreated control AGM in the efficacy study, FY07-070. The safety profile of levofloxacin is well established in adults. The most common adverse reactions include nausea, headache, diarrhea, insomnia, constipation, and dizziness in greater than 3% of subjects. Levofloxacin also shares adverse reactions common to the fluoroquinolone class of antibiotics such as tendinitis and tendon rupture. Safety information is available from over 1,500 children older than 6 months to 16 years of age. The safety profile of levofloxacin has not been fully evaluated in infants younger than 6 months and a dose is not approved for this age group. Arthralgia and arthritis are more common in children treated with levofloxacin compared to non-fluoroquinolone treated children.

Overall, the benefits of treating life-threatening pneumonic plague with levofloxacin to prevent death greatly outweigh the risks of adverse reactions from a fluoroquinolone antibacterial drug such as levofloxacin.

1.3 Recommendations for Postmarket Risk Evaluation and Mitigation Strategies

There are no recommendations for postmarket risk evaluation and mitigation strategies.

1.4 Recommendations for Postmarket Requirements and Commitments

The sponsor will be asked to design a protocol to collect and evaluate efficacy and safety information for levofloxacin for the treatment pneumonic plague. The protocol should be

submitted to the FDA for review. This protocol should be ready for implementation in the event of an intentional release of *Y. pestis* in the US population.

2 Introduction and Regulatory Background

2.1 Product Information

Levofloxacin, LEVAQUIN®, belongs to the fluoroquinolone class of antibacterial agents. LEVAQUIN® was approved by the Food and Drug Administration on December 20, 1996. There are three available formulations of levofloxacin, an oral tablet, oral solution, and an injectable solution.

FDA approved indications include:

- Pneumonia: nosocomial and community acquired
- Acute bacterial sinusitis
- Acute bacterial exacerbation of chronic bronchitis
- Skin and skin structure infections: complicated and uncomplicated
- Chronic bacterial prostatitis
- Urinary tract infections: complicated and uncomplicated
- Acute pyelonephritis
- Inhalational anthrax, post-exposure

The usual dose of LEVAQUIN® Tablets or Oral Solution is 250 mg, 500 mg, or 750 mg administered orally every 24 hours, as indicated by the type of infection. The usual dose of LEVAQUIN® Injection is 250 mg or 500 mg administered by slow infusion over 60 minutes every 24 hours or 750 mg administered by slow infusion over 90 minutes every 24 hours. See LEVAQUIN® USPI in Appendix I for further information regarding dosing and safety information.

Currently Available Treatments for Proposed Indications

Currently available FDA-approved antibacterial drugs for treatment of *Y. pestis* infection include streptomycin, doxycycline, tetracycline, and other antibacterial agents in the tetracycline group. Gentamicin is recommended in treatment guidelines on treatment of plague, but is not approved for the indication. Evidence for the efficacy of tetracyclines for the treatment of pneumonic plague is limited, therefore, additional antibiotic therapy for plague is warranted. In an effort to broaden the therapeutic armamentarium for plague, the efficacy of intravenous levofloxacin (LEVAQUIN®) in a non-human primate model of pneumonic plague, the African Green Monkey, was investigated by the National Institutes of Allergy and Infectious Diseases.

FDA-approved antibacterial drugs for treatment of plague are summarized in Table 1.

Table 1. Current Available Therapies for Human Plague

Drug -Trade Name(s)	Indication & Usage section (verbatim)	Pediatric use (limitation)	Generics (Y/N)	SNS (Y/N)
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(formulations)				
Doxycycline -Vibramycin (tablets, intravenous injection, suspension) -Monodox (capsules) -Doryx (pellet filled oral capsule)	Doxycycline is also indicated for the treatment of the following infections caused by gram-negative organisms: Plague due to <i>Yersinia pestis</i> (formerly <i>Pasteurella pestis</i>) (source Vibramycin)	Yes (for use in anthrax or pediatric patients older than 8 years of age)	Yes	Yes
Tetracycline -Achromycin (capsules) -Sumycin (tablet, capsule)	ACHROMYCIN is indicated in infections caused by the following microorganisms. <i>Yersinia pestis</i> and <i>Francisella tularensis</i> , formerly <i>Pasteurella pestis</i> and <i>Pasteurella tularensis</i>	Yes (pediatric patients older than 8 years of age)	Yes	No
Minocycline -Minocin (capsule, tablet)	Plague due to <i>Yersinia pestis</i>		Yes	No
Demeclocycline (Declomycin) (tablet)	<i>Yersinia pestis</i> , formerly <i>Pasteurella pestis</i>		Yes	No
Streptomycin [Note: for IM administration only]	Streptomycin is indicated in the treatment of individuals with moderate to severe infections caused by <i>Pasteurella pestis</i> (plague)	Yes	Yes – sole source	No

Source: review by Y. Yasinskaya MD, DAIP

2.3 Availability of Proposed Active Ingredient in the United States

Levofloxacin, LEVAQUIN®, is marketed in the United States.

2.4 Important Safety Issues

Safety was not assessed in the efficacy study in AGM model.

Safety in Humans: Pre- and post-market safety information for levofloxacin in humans has been reviewed by the FDA and is summarized in the Levaquin® label. The safety of levofloxacin has been established based on approval of existing indications of pneumonia (nosocomial and community acquired), acute bacterial sinusitis, acute bacterial exacerbations of chronic bronchitis, skin and skin structure infections (complicated and uncomplicated), chronic bacterial prostatitis, urinary tract infections (complicated and uncomplicated), acute pyelonephritis, and inhalational anthrax (post-exposure).

The product label contains the most current safety information from clinical trials and from postmarket experience. Levofloxacin, a fluoroquinolone, shares a number of serious adverse reactions with other antibacterial drugs in the class, including tendinopathy, exacerbation of myasthenia gravis, central nervous system and peripheral nervous system disorders, hypersensitivity, hepatotoxicity, QT prolongation, and *C. difficile* associated diarrhea. These serious adverse events are described in detail in the Warnings and Precautions section of the levofloxacin label. In clinical trials, the most common adverse

drug reactions (occurring $\geq 3\%$ of subjects) were nausea, headache, diarrhea, insomnia, constipation, and dizziness.

With respect to counterterrorism indications, levofloxacin received approval in November 2004 for treatment of inhalational anthrax (post-exposure) in adults. In May 2008, levofloxacin received approval for pediatric patients ≥ 6 months of age for the prevention of inhalational anthrax (post-exposure). In adult and pediatric patients, ≥ 50 kg and ≥ 6 months of age, the dose is 500 mg once daily for 60 days. In pediatric patients, < 50 kg and ≥ 6 months of age the dose is 8mg/kg Q12H not to exceed 250 mg per dose once daily for 60 days.

In clinical trials, 1,534 children (6 months to 16 years of age) were treated with oral and intravenous LEVAQUIN®. Children treated with LEVAQUIN® had a significantly higher incidence of musculoskeletal disorders (arthralgia, arthritis, tendinopathy, gait abnormality) when compared to the non-fluoroquinolone-treated children as described in *Use in Specific Populations* in the LEVAQUIN® label.

Levofloxacin labeling includes a Medication Guide for patients describing the risks of tendon rupture and worsening of symptoms of myasthenia gravis.

2.5 Summary of Presubmission Regulatory Activity Related to Submission

On December 1, 2010, a pre-sNDA meeting was held with the applicant and NIH/NIAID to reach agreement on the content of the upcoming sNDA. In February 2011, the FDA requested a stand-alone bridging pharmacokinetic study protocol in healthy African Green monkeys to be conducted to address bioanalytical deficiencies in NIAID Study FY-07-107. A new protocol for the bridging pharmacokinetic study was submitted in March 2011 which was acceptable to the FDA review team. The purpose of the pharmacokinetic studies in the development program was to demonstrate that the humanized levofloxacin dose used in the animal efficacy study provide the AGM with concentration-time profiles and systemic exposure comparable to or lower in the animals than that in humans who received an approved levofloxacin dose for treatment of other infections.

On April 29, 2011, the applicant submitted information containing definitions and mock datasets for the upcoming efficacy supplement.

The “Animal Rule”

Products for the treatment or prevention of human disease are generally studied in adequate and well-controlled clinical trials that enroll patients with the disease, and these clinical trials, along with other investigations, serve as the basis for approving or licensing the product. However, pneumonic plague is extremely rare and human trials are not feasible. The pivotal efficacy study in this supplemental NDA for levofloxacin for the treatment of pneumonic plague will be assessed for approval under the “Animal Rule”. The “Animal Rule” is a regulatory mechanism to review efficacy studies in animal

models for diseases that cannot be studied in human beings. FDA published a Final Rule in 2002 entitled “*New Drug and Biological Drug Products; Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible*,”¹ that is also referred to as “*The Animal Rule*.” Under the *Animal Rule*, products intended to reduce or prevent serious or life-threatening conditions in humans may be approved by the FDA based on evidence of effectiveness derived from controlled studies in animal models of the disease with additional supporting safety and pharmacokinetic data. This rule is summarized in the Code of Federal Regulations (CFR), in Title 21 CFR 314.600 (Subpart I) for New Drugs and in Title 21 CFR 601.90 for Biologic Products. Levofloxacin is a drug product, and therefore, the provisions of 21 CFR 314.600 (Subpart I) regulations apply.

Criteria for Submission

All animal studies subject to the Animal Rule regulations must be conducted in accordance with good laboratory practices. Products evaluated for effectiveness under Subpart I are expected to be evaluated for safety under preexisting requirements for establishing the safety of new drugs.

Criteria for Approval of a Drug under the Animal Rule

The Animal Rule states that a drug can be approved on the basis of adequate and well-controlled animal studies when the results of those animal studies establish that the biological product is reasonably likely to produce clinical benefit in humans.

To ensure applicability of animal data to human disease the following would need to be established:

- Pathophysiology of the disease and product’s mechanism of action are reasonably well understood;
- The product efficacy is demonstrated in more than one animal model, unless one animal model is sufficiently well characterized for predicting the response in humans;
- The animal study endpoint is clearly related to the desired benefit in humans; and
- Data on product pharmacokinetics and pharmacodynamics in animals and humans allow selection of an effective human dose.

In assessing the sufficiency of animal data, the FDA may take into account other data, including human data in other indications. For example, product efficacy in diseases of similar pathophysiology might provide supportive information for the indication being sought. Therefore, data from appropriate studies to address each of the above bullet points would need to be provided to support the conclusion that the product is effective.

In addition, approval under this regulation will be subject to three requirements:

1. Postmarketing studies: The applicant must conduct postmarket studies, such as field studies, to verify and describe the drug’s clinical benefit and to assess its safety when used as indicated when such studies are feasible and ethical (for example during or following a bioterrorism event).

2. Approval with restrictions to ensure safe use: If FDA concludes that a drug product shown to be effective under this regulation can be safely used only if distribution or use is restricted, FDA will require such postmarket restrictions as are needed to ensure safe use of the drug product.
3. Information to be provided to patients: For drug products or specific indications approved under this subpart, applicants must prepare, as part of their proposed labeling, an information sheet to be provided to patients that includes the following: a disclaimer that the drug's approval was based on solely animal efficacy studies, the indication, directions for use, foreseeable risks and benefits, and any other relevant information required by FDA at the time of approval.

FDA may withdraw approval if:

- A postmarket clinical study fails to verify clinical benefit;
- The applicant fails to perform the postmarket study with due diligence;
- Use after marketing demonstrates that postmarket restrictions are inadequate to ensure safe use of the drug product;
- The applicant fails to adhere to the postmarket restrictions applied at the time of approval under this subpart;
- The promotional materials are false or misleading; or
- Other evidence demonstrates that the drug product is not shown to be safe or effective under its conditions of use.

2.6 Other Relevant Background Information

Not applicable.

3 Ethics and Good Clinical Practices

The efficacy study in AGM was conducted in accordance with good laboratory practices (GLP).

3.1 Submission Quality and Integrity

The quality and integrity of the application is acceptable.

OSI conducted an audit at [REDACTED] (b) (4). This inspection at [REDACTED] (b) (4) was focused on the complete bioanalytical portion of the studies for analyses of levofloxacin in African Green Monkey plasma. With regard to the levofloxacin study, [REDACTED] (b) (4) Study No. B122-03: *A Pharmacokinetic and Toxicity Study of Levofloxacin Following Intravenous and Oral (Nasogastric) Administration to African Green Monkey (uninfected)*, the inspectional observations were documentation and recording issues, and were not considered to have an adverse impact on the study results. OSI recommended that the submitted pharmacokinetic data were acceptable for review.

An FDA inspection was conducted at the Lovelace facility in August/September 2011 and an EIR report is pending.

3.2 Compliance with Good Clinical Practices

Not Applicable – no human clinical studies were submitted.

3.3 Financial Disclosures

There were no clinical studies required to support this NDA therefore no financial disclosures were provided.

4 Significant Efficacy/Safety Issues Related to Other Review Disciplines

Not applicable.

4.1 Chemistry Manufacturing and Controls

Not applicable.

4.2 Clinical Microbiology

See clinical microbiology review by Simone Shurland, Ph.D.

4.3 Preclinical Pharmacology/Toxicology

Based on the results of the four natural history studies of plague in the AGM model, this species was selected for the efficacy study of levofloxacin as a treatment of pneumonic plague.

In the natural history studies, fever was the clinical sign most consistently associated with onset of plague and onset of fever was chosen as a trigger for initiation of antibiotic treatment in the efficacy study, FY-07-070. Findings in the four natural history studies are summarized in Table 2. These studies were previously reviewed by Yuliya Yasinskaya, MD and Ariel Porcala, MD.

Table 2. Summary of Findings in the Four Natural History Studies in AGM

Parameter	Battelle Study 617-G607610	Battelle Study 875-G607610	LLRI	USAMRIID
Average <i>Y. pestis</i> Exposure Dose of (range)	614 LD ₅₀ (106 LD ₅₀ to 1150 LD ₅₀)	48 LD ₅₀ (24 LD ₅₀ to 88 LD ₅₀)	134 LD ₅₀ (44 LD ₅₀ to 255 LD ₅₀)	9 LD ₅₀ to 57 LD ₅₀
Animals	3 males and 7 females	5 males and 5 females	5 males and 5 females	3 males and 3 females
Mortality	100% 10 found dead	100% 10 found dead	100% (3 found dead, 7 euthanized)	67% (4/6: 1 found dead, 3 euthanized)
Average Time to Death (range)	72 hours (61 to 90 hours)	93.5 hours (67.5 to 139 hours)	90 hours (72 to 100 hours)	112 hours (99.5 to 125 hours)
Average Time to Fever Onset (range)	44 hours (39 to 49.5 hours)	54 hours (47 to 61.5 hours)	64 hours (46 to 80.5 hours)	73 hours (70 to 76 hours)
Average Interval between Fever and Death (range)	28.5 hours (16 to 44.5 hours)	39 hours (17.5 to 78 hours)	26 hours (16 to 42 hours)	38.5 hours (29.5 to 51 hours)
Initial Level of Bacteremia (cfu)	33 to >650,000 cfu/mL	600 cfu to >7,000,000 cfu/mL	10 cfu to >200,000 cfu/mL	3 x 10 ⁶ to 9 x 10 ⁸ cfu/mL (terminal)
Timing of Bacteremia Onset (range)	48 to 72 hours (100% + by Day 2)	43.5 hours (30 to 68.5 hours)	66.5 hours (43 to 94 hours)	60 hours (48 to 72 hours)
Radiographic abnormalities	n/a	n/a	5/10 on Day 3	4/4 at 80 - 83h post exposure
Symptoms	Fever, loss of appetite, respiratory distress, bloody respiratory secretions, lethargy	Fever, hunched posture, respiratory distress, bloody respiratory secretions, lethargy	Fever, loss of appetite, diarrhea, lethargy	Fever, loss of appetite, respiratory distress, lethargy
Pathology	Hemorrhagic pneumonia	Hemorrhagic pneumonia	Fibrino-suppurative hemorrhagic pneumonia	Fibrino-suppurative hemorrhagic pneumonia

Source: Review by Y. Yasinskaya, M.D.

4.3.1 Comparison of the AGM model of pneumonic plague model with human pneumonic plague

The AGM model of pneumonic plague is similar to the human pneumonic plague with respect to the time course of the disease, the time to onset of fever post-exposure, mortality, chest radiographic findings, and pulmonary histopathology, Table 3. The comparison is based on information in a limited number of cases available in published literature.

Table 3. Comparison of Pneumonic Plague in Humans and in the African Green Monkey model of Pneumonic Plague

Table 1: Human and African Green Monkey Natural Courses of Pneumonic Plague

	Human^a	African Green Monkey^b
Time course of disease, days	2 to 9	2 to 9
Temperature	Elevated in ~100% of cases (at 3 days in 1 case)	Elevated in 100% of cases (typically 3 days post-exposure)
<i>Yersinia pestis</i> present	Positive in 100% of sputum	Positive in 100% of blood and lung/nasal fluids
Heart rate	Elevated	Elevated
Respiration rate	Elevated late in disease	Elevated late in disease
Chest radiographs	Pulmonary infiltrates, 90% bilateral	Pulmonary infiltrates, 80% bilateral
Pathology, lung	Consolidations, Inflammatory infiltrates, Hemorrhagic/frothy fluid, Exudates and effusions, Bronchopneumonia, Bacilli	Bacteria, Edema, Hemorrhage, Inflammatory infiltrates/bronchopneumonia, Pleural fibrin

^a Data from 3 cases in 3 publications [8,16,17](#)

^b Data from 41 untreated AGMs from 5 studies ([Mod4.2.1.1\F03-09G](#), [FY06-126](#), [617-G607610](#), [875-G607610](#), and [FY07-070](#))

Source: Applicant's Study Report, Study FY07- 070

4.3.2 Study No. FY07-070: Pivotal Efficacy Study in AGM Model of Pneumonic Plague

Study FY07-070 was conducted at LBERI during 2008 - 2009. The study was randomized, investigator-blinded, treatment study of levofloxacin versus placebo in AGM exposed to aerosolized *Y. pestis*. Three cohorts (Cohorts 1, 2, and 3) of AGM were studied. The objective of the study was to determine if intravenous infusion of levofloxacin would prevent death from established pneumonic plague in the AGM model.

A total of 26 AGM were selected for the study. Animals were assorted into test groups based on bodyweights using a validated computerized data acquisition system. Animals were randomized into housing placement and exposure order using a random number generator. Two animals were removed from the study, one animal due to health reasons immediately post randomization (levofloxacin group) and one animal (post randomization from the levofloxacin group) due to a protocol deviation because levofloxacin treatment was initiated prior to development of fever. A total of 24 animals, 12 males and 12 females, remained in the study.

Each cohort of African Green monkeys (*Chlorocebus aethiops*) was challenged via head-only aerosol inhalation to achieve a target dose of 100 ± 50 LD₅₀ of *Y. pestis*, strain CO92. Based on the results of the natural history studies in AGM, established pneumonic plague was indicated by a body temperature of a mean fever $>39^{\circ}\text{C}$ for at least one hour, which was the trigger to initiate levofloxacin or placebo infusions.

Following challenge with aerosolized *Y. pestis*, the animals were monitored via telemetry to determine the onset of defined fever. The animals were treated with levofloxacin or placebo within six hours (3.4 ± 1.8 hours) of onset of fever with levofloxacin (5 mg levofloxacin/mL in 5% dextrose), or 5% dextrose, depending on predetermined treatment group. Eighteen AGM (10 females and 8 males) received levofloxacin and seven AGM (3 females and 4 males) received placebo. One animal (X779, female) received one dose of levofloxacin and was removed from the study for a protocol deviation leaving 17 AGM in the levofloxacin group.

For each animal, the infusion of levofloxacin was administered as a "humanized" dose regimen consisting of two infusions every 24 h period in order to target plasma levels achieved in humans with a single dose of levofloxacin 500mg every 24 h. Levofloxacin was administered for 10 days. In each 24 h period, levofloxacin 8 mg/kg (high dose) or control article was administered over 30 minutes followed by a second infusion of levofloxacin [2 mg/kg (low dose)] or control article administered over 30 ± 8 minutes within $12 (\pm 1.0)$ h. Infusions continued until the death of the animal or until 20 total infusions (10 high doses and 10 low doses) had been delivered.

Animals were monitored for up to 28 days post-challenge by twice daily observations and continuous monitoring of heart rate, respiration rate, and temperature via implanted telemeters.

Baseline telemetry data were collected pre-challenge along with body weight, hematology, clinical chemistry, bacterial blood culture information, plasma levofloxacin concentration, and chest radiographs. For Cohorts 1 and 2, arterial blood gas, blood coagulation parameters and echocardiography were also measured.

Following euthanasia or spontaneous death, all animals were weighed, blood was collected for bacterial culture, hematology, and clinical chemistry parameters, and a gross necropsy was performed. Select tissue samples of lung (lesion and non-lesion) and liver, spleen, kidney, and brain were collected for bacterial quantitation and histopathological examination.

Study Population (Test Animals)

The population included AGM (*Chlorocebus aethiops*) jacketed with implanted telemeters and intravenous catheters. Animals were conditioned to a restraint collar, poles, restraint chairs, and limb restraints. Room temperature was 18-29 °C. The animals were received from the National Institutes of Health c/o Alpha-Genesis, Inc. (Yemassee, SC). The animals weighed approximately 3 - 8 kg and were at least two years old when assigned to the study. The animals were wild-caught therefore, actual ages were unknown. Animal rooms and cages were cleaned and sanitized prior to use. Animals were individually housed in stainless steel cages with wire mesh bottoms. Animals were transferred to sanitized cages every two weeks, except for one protocol deviation. Drinking water was provided ad libitum. Animal rooms were maintained on a 15-hour light/9-hour dark cycle beginning at least one week prior to exposure and during the assay period.

Implantation of Study Devices

Animals received surgically implanted telemetry monitoring devices, then intravenous catheters, and then moved into the BSL-3 at least one week prior to challenge with *Y. pestis*. Telemeters were surgically placed on the left abdominal wall to provide continuous monitoring of body temperature, intrathoracic pressure, respiratory rate, heart rate, and basic electrocardiographic signal traces detecting clinically significant arrhythmias.

All animals had IV catheters [Broviac (Cohorts 1 and 2) or Hickman [dual-port (Cohort 3)] inserted in the right femoral vein. The catheter was tunneled through the right flank and back, emerging through the skin of the upper mid-back. The exit site and catheter were protected by a jacket. No study animals received systemic antibacterial drugs within 28 days prior to aerosol exposure with *Y. pestis*. Animals were identified by tattoo, jacket number, telemetry frequency, and cage card.

Challenge Agent

All work done with *Y. pestis* CO92 strain was carried out under BSL3 conditions. The *Y. pestis* CO92 strain was originally isolated from a fatal human case of pneumonic plague (standard F1 positive CO92 strain used for plague studies at USAMRIID). The CO92 strain has antibacterial MIC comparable to other plague strains collected from 1977 to 1998 by the California Microbial Diseases Laboratory. The cut-off of > 20 LD₅₀ that

caused fatal pneumonic plague was previously described in an inhalational plague study in AGM by USAMRIID.

Preparation of Material for Aerosolization

Following centrifugation of a suspension of *Y. pestis*, the cell pellet was suspended and the optical density (OD) was determined. The bio-aerosol sprays were prepared from the suspended centrifuged culture and a previously prepared concentration/OD curve. The suspended culture was adjusted as required to achieve the target aerosol exposure level.

Aerosolized *Y. pestis*

Nebulizer solutions of *Y. pestis* strain CO92 were formulated for each cohort on Study Day 0. The target particle size was 1 to 3 μm . On Day 0, all study animals were exposed to aerosolized *Y. pestis* at an approximate target dose of 100 LD₅₀ [actual range: 3-145 x LD₅₀].

Test Drugs

The test article for this study was levofloxacin, Levaquin® Injection Premix in Single-Use Flexible Containers (5 mg levofloxacin/mL 5% dextrose). The control article was 5% Dextrose (in water) for Injection.

Levofloxacin Dose

The trigger to start treatment with intravenous levofloxacin or placebo was the onset of fever defined as a mean body temperature $> 39^{\circ}\text{C}$ for more than one hour recorded by telemetry.

In each individual animal, an infusion of levofloxacin, 8 mg/kg (high dose) or control article was administered over 30 ± 5 minutes within every 24 ± 0.5 h. To mimic the human pharmacokinetics of levofloxacin, a second infusion of levofloxacin, 2 mg/kg (low dose), or control article, was administered over 30 ± 5 minutes within 12 h after the 24 h (i.e., high dose) infusion. Infusions continued until the death of the animal or until 20 total infusions (10 high doses and 10 low doses) had been delivered.

Study Procedures

All animals were anesthetized prior to handling for physical examinations, chest radiographs and aerosol exposure. Pre-exposure, all animal activity was observed daily. Body temperature data were collected by continuous monitoring via telemetry. Baseline temperature and telemetry data were collected for approximately seven days prior to exposure to aerosolized *Y. pestis*.

Clinical observations (general appearance, posture, and movement) and animal behavior were performed at intervals. Arterial blood gases were done for some cohorts. Blood cultures were drawn once pre-study, daily during Day 2 to Day 6 post-exposure, and at intervals during the study. Hematology, chemistry, electrolytes, renal and liver function were monitored. Echocardiography was performed for each animal. Chest radiographs were done pre-exposure and post-exposure in Cohorts 1 and 2 but not in Cohort 3. A terminal necropsy was performed for each euthanized animal (survivors) at Day 28 and interim necropsies were performed on animals that died or were euthanized prior to the end of the study. Histopathological analyses were performed on lung tissue and other

organs. Levofloxacin concentrations for pharmacokinetic (PK) analyses were measured throughout the study.

The investigator charged with making decision regarding euthanasia was blinded to treatment allocation. Criteria for euthanasia are summarized in the following table.

Criteria for Euthanasia	
Observation	Threshold for Euthanasia
Respiratory Rate by telemetry	>60 breaths per min
Heart Rate by telemetry and EKG	> 200 beats/min or abnormal repolarization
Respiratory character by observation	Labored breathing with excessive work of breathing
Behavior by observation and activity monitor	Any seizures, OR too weak to climb onto perch falling off perch OR constant hunched position and unresponsive to stimulation and refusal to eat any offered food

4.4 Clinical Pharmacology

4.4.1 Mechanism of Action

Levofloxacin belongs to the fluoroquinolone class of antibacterial agents. Fluoroquinolones directly inhibit DNA synthesis through the interaction of the drug complexes with one or both of the bacterial type II topoisomerase enzymes, DNA gyrase and topoisomerase IV. The type II topoisomerases play essential roles in bacterial DNA replication, transcription, repair, and recombination.

Although in vitro microbiological data suggest that levofloxacin at a dose of 500 mg once daily (QD) is an appropriate dose to evaluate for the treatment of plague in humans, evaluation of the efficacy of levofloxacin in the treatment of pneumonic plague cannot be conducted directly in humans due to ethical concerns and must rely on the results of animal models. Thus, the efficacy of levofloxacin in the treatment of pneumonic plague was evaluated with a humanized dose in African Green Monkeys (AGM). Accordingly, the purpose of the pharmacokinetic studies in the applicant's development program was to demonstrate that the humanized levofloxacin dose used in the animal efficacy study provides the AGM with concentration-time profiles and systemic exposure comparable to or lower in the animals than that in humans who received an approved levofloxacin dose for treatment of other infections.

See review by Seong Jang, Ph.D. for analysis of the studies conducted to compare the pharmacokinetics (PK) of levofloxacin in African Green Monkeys and the PK of levofloxacin in healthy human volunteers.

4.4.2 Pharmacodynamics

There is no new information on pharmacodynamics

4.4.3 Pharmacokinetics

Levofloxacin plasma concentrations in humans exceed the MIC against *Y. pestis* (0.03 mcg/mL) for the entire dosing interval following the administration of 500 mg QD via IV infusion. Since the $t_{1/2}$ of levofloxacin in AGM is shorter than in humans (approximately 2 to 3 hours vs. 6 to 8 hours), a PK simulation was conducted to determine a humanized dosing regimen in order to mimic the human plasma profile of levofloxacin in AGM. The simulation results showed that a 30 minute IV infusion of 8 mg/kg followed by an additional 30 minute IV infusion of 2 mg/kg given 12 hour later (8/2 mg/kg) would provide AGM with a levofloxacin plasma concentration-time profile comparable to that in humans receiving the 500 mg IV once daily. This humanized dose regimen was used in this efficacy study in AGM (Study FY07-070).

The 8/2 mg/kg dose regimen kept systemic exposure of levofloxacin in AGM, in the efficacy study, lower than that in humans receiving 500 mg IV once daily.

4.4.4 Discussion of Individual Studies/Clinical Trials

There are no clinical studies in these NDA efficacy supplement submissions

5.0 Sources of Clinical Data

5.1 Tables of Studies/Clinical Trials

There are no clinical studies in these NDA efficacy supplement submissions. See section 4.3 for summary of the pivotal efficacy study in the African Green Monkey model of pneumonic plague.

5.2 Review Strategy

Not applicable

5.3 Discussion of Individual Studies/Clinical Trials

Not applicable

6 Review of Efficacy

The primary efficacy endpoint in Study FY07-070 was survival at end of study, Day 28. The primary analysis population was conducted in the intent-to-treat (ITT) population. The efficacy results for the ITT population are shown in Table 4. Sensitivity analyses are presented in Tables 5, 6, and 7.

Analyses of survival were performed on the following populations:

Intention-to-treat (ITT) Population: Animals that received the aerosol challenge, but died before or during treatment with placebo or active drug, were included in this population as treatment failures. A conservative approach was taken to the analysis of survival i.e., animal X779 which was withdrawn from the study post-randomization for a protocol deviation and survived to Day 28 is not included in the analysis.

Survival in the ITT population: Levofloxacin-Treated versus Placebo-Treated Animals
None of the control animals survived, all were dead or euthanized by Day 5. Three of the control animals (U193, X762, X773) were euthanized moribund and four animals died spontaneously. A total of 16/17 (94%) levofloxacin-treated animals survived to the end of the study at Day 28 compared to 0% of controls which was a statistically significant result, Table 4.

Table 4. Survival in ITT Population

	Levofloxacin*	Placebo
Survival (%)	16/17 (94%)	0/7
95% CI	55.5%, 99.9%	
P value	<0.0001	

* Animal X779 was removed from the study post-randomization for a protocol deviation and survived and is not included in this analysis. If X779 is included as non-survivor, the result remain statistically significant, 95% CI [42.1%, 98.6%], p-value <0.0001.
Fisher's exact test (two-sided), exact 95% CI for difference in survival rates was calculated.

The clinical findings in survivors were consistent with resolving pneumonia, for example animals had resolution of high fever and decrease in respiratory rates after 2 to 3 days on levofloxacin treatment. Survivors appeared clinically well at the end of the study. Additionally, resolution of pulmonary infiltrates was observed in the levofloxacin-treated animals that had follow-up chest x-rays (n=6) at the end of study, i.e. Day 28. The histopathological findings in levofloxacin-treated animals were consistent with resolving pneumonia.

All control animals succumbed to pneumonic plague. The histopathological findings of fibrino-suppurative pneumonia in control animals were similar to the pulmonary histopathological findings described for pneumonic plague in the natural history studies in AGM.

6.1 Indication

The proposed indication is the treatment of pneumonic plague in adults and children ≥ 6 months of age.

6.1.1 Methods

The study report for Study FY-07-070 was reviewed and is described in Section 10. Analyses of raw data were performed using the review tool, JReview 9.2. See complete review of the pivotal efficacy, Study FY07-070 in Section 10.

6.1.3 Subject Disposition

Twenty-six AGM were enrolled in the Study FY07-070. Two animals in the levofloxacin-treatment group were withdrawn from the study. Animal, X717 in the levofloxacin treatment group was withdrawn from the study for illness post-randomization but it did not receive a challenge dose of *Y. pestis*, was not treated with levofloxacin, and is not included in the ITT analysis. In the levofloxacin-treatment group, one animal, X779, was removed from the study post-exposure to *Y. pestis* for a protocol deviation - it received a first dose of levofloxacin prior to development of fever. Twenty-four animals (12 males and 12 females) remained in the study.

6.1.4 Analysis of Primary Endpoint(s)

The primary endpoint was survival at the end of the study, Day 28.

6.1.5 Analysis of Secondary Endpoints(s)

Not applicable

6.1.6 Other Endpoints

Microbiologic clearance of blood/tissue cultures was evaluated. *Y. pestis* was isolated from blood and or tissue culture in three survivors. Two of the 16 (12.5%) survivors in the levofloxacin treated group had evidence of low colony counts of *Y. pestis* in their blood cultures at the end of the study, Day 28. No follow-up was possible for these animals as they were euthanized per protocol at the end of the study.

6.1.7 Subpopulations

The following subpopulations of AGM were evaluated for survival at end of study, Day 28.

Animals that received a challenge dose > 20 LD₅₀: A cut of > 20 LD₅₀ was chosen because two animals that were untreated and received ≤20 LD₅₀ *Y. pestis* survived in a natural history study of pneumonic plague in the AGM model.

Animals that were bacteremic (Y. pestis) at treatment: Animals that were bacteremic (evidenced by a positive blood culture) at the time of therapeutic intervention.

Animals that had radiographic evidence of pneumonia: Animals that had evidence of pulmonary infiltrates on chest radiograph consistent with pneumonia.

6.1.8 Analysis of Clinical Information Relevant to Dosing Recommendations

See Levofloxacin USPI, Appendix I

6.1.9 Discussion of Persistence of Efficacy and/or Tolerance Effects

Not applicable

6.1.10 Additional Efficacy Issues/Analyses

Analyses of survival were conducted in sub-populations of AGM in Study FY07-070. In addition to the primary analysis of survival in the ITT population, survival was assessed in animal that received challenge doses > 20 LD₅₀, bacteremic animals, and animals with radiologic evidence of pneumonia. In all subpopulations that were evaluated, the survival rate in levofloxacin-treated animals was significantly greater than the survival rate in placebo animals.

Animals that received a challenge dose > 20 LD₅₀ *Y. pestis*

All animals in Cohort 1 and Cohort 2 received challenge doses > 20 LD₅₀ of *Y. pestis*. This cut-off of 20 LD₅₀ was chosen based on the natural history studies, which demonstrated that animals that succumbed to challenge with *Y. pestis* CO92 strain had > 20 LD₅₀. Six animals in the levofloxacin-treated group in Cohort 3 received a challenge dose of 3 to 12 LD₅₀ and these animals are excluded from this analysis. All control animals received a challenge dose > 20 LD₅₀. The two control animals in Cohort 3 received challenge doses of 47 LD₅₀ and 48 LD₅₀, respectively.

Table 5. Survival in Animals with > 20 LD₅₀

	Levofloxacin	Placebo
Survival (%)	11/11 (100%)*	0/7
95% CI	58.9%, 100%	
P value	<0.0001	

*Six levofloxacin-treated animals that received a challenge dose of < 20 LD₅₀ are excluded from analysis however all became bacteremic. Five of these animals survived to the end of the study, Day 28

Animals that were Bacteremic at Start of Treatment

Five levofloxacin-treated animals (one in cohort 1; three in cohort 2; one in cohort 3) did not have positive blood cultures at the start of treatment and are excluded from the following analysis. The survival rate was 92% among levofloxacin-treated animals that were bacteremic at the time of treatment. The animals that received a challenge dose < 20 LD₅₀ were bacteremic with *Y. pestis* and are included in this analysis.

Table 6. Survival - Animals with bacteremia at start of treatment

	Levofloxacin	Placebo
Survival (%)	11/12 (91.7%)*	0/5†
95% CI	28.0%, 99.8%	
P value	<0.001	

*One levofloxacin-treated animal (#Y293) had 1 colony of *Y. pestis* isolated from a pre-infusion blood sample on a qualitative culture plate on Day 3, all other catheter blood plates at this time point were contaminated with colonies inconsistent with *Y. pestis*. If Animal Y293 was included in the analysis, the survival is 92% (12/13), 95% CI (28.3%, 99.8%), P value 0.0007

†Two control animals (X773 and X888) were not bacteremic prior to first infusion. They became bacteremic the day after the first infusion.

Animals with Radiologic Evidence of Pulmonary Infiltrates

All animals in Cohorts 1 and 2 that had chest radiographs performed post-exposure to *Y. pestis* had evidence of pulmonary infiltrates. Animals in Cohort 3 had baseline, pre-exposure chest radiographs only and are excluded from the analysis. Two of five animals in the control group in cohorts 1 & 2 died or were euthanized before chest x-rays could be performed. The survival rate in these animals that had pulmonary infiltrates was 100% compared to 0% in control animals.

Table 7. Animals with pulmonary infiltrates on chest radiograph (cohorts 1 and 2)

	Levofloxacin	Placebo
Survival (%)	9/9 (100%)	0/3
95% CI	29.0%, 100%	
P value	0.005	

7 Review of Safety

Safety information is not evaluated in Study FY07-070. Study FY07-070 is an efficacy study conducted in the AGM model to evaluate levofloxacin IV treatment versus placebo for pneumonic plague.

7.1 Methods

7.1.1 Studies/Clinical Trials Used to Evaluate Safety

Not applicable

7.1.2 Categorization of Adverse Events

Not applicable

7.1.3 Pooling of Data Across Studies/Clinical Trials to Estimate and Compare Incidence

Not applicable

7.2 Adequacy of Safety Assessments

7.2.1 Overall Exposure at Appropriate Doses/Durations and Demographics of Target Populations

Not applicable

7.2.2 Explorations for Dose Response

Not applicable

7.2.3 Special Animal and/or In Vitro Testing

Not applicable

7.2.4 Routine Clinical Testing

Not applicable

7.2.5 Metabolic, Clearance, and Interaction Workup

Not applicable

7.2.6 Evaluation for Potential Adverse Events for Similar Drugs in Drug Class

Not applicable

7.3 Major Safety Results

7.3.1 Deaths

See efficacy analysis for primary endpoint of survival at end of study, Day 28.

7.3.2 Nonfatal Serious Adverse Events

Not applicable

7.3.3 Dropouts and/or Discontinuations

Not applicable

7.3.4 Significant Adverse Events

Not applicable

7.3.5 Submission Specific Primary Safety Concerns

Not applicable

7.4 Supportive Safety Results

Not applicable

7.4.1 Common Adverse Events

Not applicable

7.4.2 Laboratory Findings

Not applicable

7.4.3 Vital Signs

Not applicable

7.4.4 Electrocardiograms (ECGs)

Not applicable

7.4.5 Special Safety Studies/Clinical Trials

7.4.6 Immunogenicity

Not applicable

7.5 Other Safety Explorations

7.5.1 Dose Dependency for Adverse Events

Not applicable

7.5.2 Time Dependency for Adverse Events

Not applicable

7.5.3 Drug-Demographic Interactions

Not applicable

7.5.4 Drug-Disease Interactions

Not applicable

7.5.5 Drug-Drug Interactions

Not applicable

7.6 Additional Safety Evaluations

Not applicable

7.6.1 Human Carcinogenicity

Not applicable

7.6.2 Human Reproduction and Pregnancy Data

Not applicable

7.6.3 Pediatrics and Assessment of Effects on Growth

Not applicable

7.6.4 Overdose, Drug Abuse Potential, Withdrawal and Rebound

See levofloxacin, Levaquin® USPI.

7.7 Additional Submissions / Safety Issues

Not applicable

8 Postmarket Experience

The safety profile of levofloxacin is well established. 7,537 patients have been exposed to levofloxacin in 29 pooled phase 3 clinical trials. The most common adverse drug reactions ($\geq 3\%$): nausea, headache, diarrhea, insomnia, constipation, and dizziness. An estimated (b) (4) treatment courses were dispensed in 2010 and (b) (4) treatment courses were dispensed in 2009. Levofloxacin shares toxicities with other antibacterial drugs in the fluoroquinolone class. The drug label includes boxed warnings for musculoskeletal events i.e., tendinitis, tendon rupture and exacerbation of muscle

weakness in patients with myasthenia gravis. Other adverse reactions include, hypersensitivity central nervous system disorders, such as convulsions, psychoses, increased intracranial pressure/pseudotumor cerebri, seizures, peripheral neuropathy, QT interval prolongation, hepatitis, blood glucose disturbance, photosensitivity, and *C. difficile* associated diarrhea. Other rare adverse reactions include, severe dermatologic reactions such as toxic epidermal necrolysis (TEN) and Steven Johnson syndrome (SJS), allergic pneumonitis, interstitial nephritis, severe hepatitis, sometimes fatal, and hematologic toxicities (agranulocytosis, thrombocytopenia).

In the pediatric population, 1,534 pediatric patients, 6m -16 years of age were exposed to levofloxacin. Clinically significant adverse reactions included musculoskeletal disorders in 2 to 3.5% of the 1,340 children followed. Arthralgia in weight bearing joints was the most common adverse reaction in pediatric patients. Arthritis, tendinopathy, and gait abnormalities were also reported. These musculoskeletal disorders resolved in all patients without sequelae and 80% of them resolved within 2 months.

Sources: Levaquin® USPI; Applicant's study report for Study FY07-070.

9 Appendices

9.1 Literature Review/References

See references in footnotes.

9.2 Labeling Recommendations

The labeling changes proposed by the applicant are under review in DAIP and are not available for inclusion at the time of writing this review.

9.3 Advisory Committee Meeting

An Advisory Committee Meeting to discuss this new drug application is scheduled for April 4th, 2012.

Questions for Levofloxacin Advisory Committee

1. Do the animal model results provide substantial evidence of effectiveness of levofloxacin for treatment of humans with pneumonic plague? If not, what additional studies are needed?

2. The safety of levofloxacin has been established based on studies and post-marketing information in existing indications. Are there any additional comments or further recommendations for safety evaluations in humans? If so, what are these recommendations?

10 Review of Individual Studies: Study FY07-070

Background

Currently available FDA-approved antibacterial drugs against *Y. pestis* infection include streptomycin, doxycycline, tetracycline, and other antibiotics in the tetracycline group. However, the supply of streptomycin is limited in the US due to infrequent use. Evidence for the efficacy of tetracyclines for the treatment of plague is limited. Gentamicin is used in clinical practice to treat plague but is not approved for the indication. Therefore, in an effort to broaden the therapeutic armamentarium for plague, the applicant investigated the efficacy of intravenous levofloxacin (LEVAQUIN®) in a non-human primate model of pneumonic plague.

Efficacy studies have been conducted in animal models of plague because naturally-occurring pneumonic plague infection in humans is rare (< 5% cases of plague) and intentional exposure to *Y. pestis* in human trials is obviously unethical.

Human Disease

Naturally occurring plague is a zoonosis primarily affecting rodents. The etiological agent of plague is *Y. pestis*. The vector is the rodent flea. Man is an incidental host. Disease forms of plague include, bubonic (80 - 95%), septicemic (10 -15%), and pneumonic (< 5%), and pharyngeal (< 5%) plague. Septicemia, meningitis may occur with bubonic and pneumonic forms.

The inhalational route of exposure causes pneumonic plague. The incubation is usually from 1 to 3 days post exposure.

Humans acquire plague via bites of rodent fleas, scratches or bites from infected domestic cats, direct handling of infected animal tissues, inhalation of respiratory secretions from infected animals, inhalation of aerosolized droplets from infected humans, or by laboratory exposure. Areas of enzootic plague include North America (mainly the southwestern United States and Pacific coastal area), the former Soviet Union, and foci in Africa, Asia, and South America. In 2000 and 2001, more than 95 percent of reported cases were from Africa, including approximately 40 percent from Madagascar.²

² Human Plague in 2000 and 2001. Wkly Epidemiol Rec. 2003;78(16):130

The World Health Organization (W.H.O.) reports approximately 1,000 to 3,000 human cases of plague/year, worldwide. From 1994 to 2003, a total of 28,530 cases of plague were reported by the WHO. In the USA, the CDC reports 10 to 20 cases/yr (average of 13 cases per year) mainly from the South West and Pacific Coast. From 1972 to 2002, the CDC reported 368 cases of human plague.

Clinical signs and symptoms include fever, cough, dyspnea, and chest pain. Chest radiographic findings include unilateral or bilateral infiltrates, consolidation, or cavities. Histopathologic findings in infected lung tissue include fibrino-suppurative pneumonia, and necrosis. Pneumonic plague is a highly lethal condition. Error! Bookmark not defined.

Y. pestis is categorized by the CDC as a Category A biological threat agent. *Y. pestis* is a potential bioweapon and could be used intentionally to create a mass-casualty event. *Y. pestis* is easily disseminated via aerosolization and inhalation by humans would cause pneumonic plague.³ Secondary pneumonic plague can occur from hematogenous spread, for example, from an infected bubo. The proposed indication in this supplemental new drug application for levofloxacin is pneumonic plague.

*Treatment Recommendations for Plague*⁴

Contained Exposure

- Streptomycin* 1 gm IM twice daily for 10 days OR
- Gentamicin, 5 mg/kg IM/IV once daily or 2 mg/kg loading dose followed by 1.7 mg/kg IM or IV 3 times daily for 10 days
- Alternative: Doxycycline* IV (FDA approved) or Ciprofloxacin IV or Chloramphenicol IV

Mass Casualty Exposure

- Doxycycline*, 100 mg PO twice daily for 7 day OR
- Ciprofloxacin, 500 mg PO twice daily for 7 days
- Alternative: Chloramphenicol

* FDA approved for treatment of plague

Case Reports of Levofloxacin Treatment of Pneumonic Plague

Published case reports on levofloxacin use for the treatment of plague are limited. There are three case reports of patients treated with levofloxacin in combination with other antibacterial drugs for the treatment of plague acquired in the United States. Two of the patients (a 39-year-old male and a 28-year-old female) with septicemic plague responded to a combination of gentamicin/ doxycycline/ levofloxacin/ ciprofloxacin and gentamicin/

³ Centers for Disease Control website: <http://emergency.cdc.gov/agent/agentlist-category.asp>

⁴ http://www.cidrap.umn.edu/cidrap/files/22/plague_clinical_pathway.pdf

levofloxacin, respectively.⁵ The third patient was a 79-year-old female with septicemic plague who initially received ampicillin-sulbactam/levofloxacin for community acquired pneumonia followed by a combination of meropenem/gentamicin when the diagnosis of plague was established. The patient developed renal failure and died.⁶

The AGM model of pneumonic plague is similar to the human pneumonic plague with respect to the time course of the disease, the time to onset of fever after exposure, mortality, chest radiographic findings, and pulmonary histopathology. The comparison is based on information in a limited number of cases (=3) available in the published literature.

Regulatory Background

Products for the treatment or prevention of human disease are generally studied in adequate and well-controlled clinical trials that enroll patients with the disease, and these clinical trials, along with other investigations, serve as the basis for approving or licensing the product. However, pneumonic plague is an example of a human disease that is rare and human trials are not feasible. In situations where human efficacy trials cannot be conducted because it would be unethical to deliberately expose healthy human volunteers to a lethal or permanently disabling toxic biological, chemical, radiological, or nuclear substance, and field trials to study the product's efficacy after an accidental or hostile exposure to the agent have not been feasible, efficacy of therapeutic products need to be derived from controlled trials in animal models of the disease, along with additional supporting data.

The FDA provides a regulatory mechanism to review efficacy studies in animal models for diseases that cannot be studied in human beings. FDA published a Final Rule in 2002 titled “*New Drug and Biological Drug Products; Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible*,”¹ that is also referred to as “*The Animal Rule*.” Under *The Animal Rule*, products intended to reduce or prevent serious or life-threatening conditions in humans may be approved by the FDA based on evidence of effectiveness derived from controlled studies in animal models of the disease with additional supporting safety and pharmacokinetic data.

This rule is summarized in the Code of Federal Regulations (CFR), in Title 21 CFR 314.600 (Subpart I) for New Drugs and in Title 21 CFR 601.90 for Biologic Products. Levofloxacin is a drug product, and therefore, the provisions of 21 CFR 314.600 (Subpart I) regulations apply. Excerpts and summaries from the Final Rule and the CFR are

⁵ MMWR September 1, 2006 / Vol. 55 / No. 34

⁶ Margolis DA. Case Report: Septicemic Plague in a Community Hospital in California *Am J Trop Med Hyg.* 2008 June; 78(6): 868–871.

provided in a separate document, "Animal Model Development to Evaluate Treatments of Pneumonic Plague" and will be discussed in a morning session of the Anti-Infective Drugs Advisory Committee (AIDAC) on April 3, 2012 and describe the type of evidence and information that need to be provided to the FDA for determination of efficacy.

Criteria for Submission: All animal studies subject to the Animal Rule regulations must be conducted in accordance with good laboratory practices. Products evaluated for effectiveness under Subpart I are expected to be evaluated for safety under preexisting requirements for establishing the safety of new drugs.

Criteria for Approval of a Drug under the Animal Rule: The Animal Rule states that a drug can be approved on the basis of adequate and well-controlled animal studies when the results of those animal studies establish that the biological product is reasonably likely to produce clinical benefit in humans.

To ensure applicability of animal data to human disease the following would need to be established:

- Pathophysiology of the disease and product's mechanism of action are reasonably well understood;
- The product efficacy is demonstrated in more than one animal model, unless animal model is sufficiently well characterized for predicting the response in humans;
- The animal study endpoint is clearly related to the desired benefit in humans; and
- Data on product pharmacokinetics and pharmacodynamics in animals and humans allow selection of an effective human dose.

In assessing the sufficiency of animal data, the FDA may take into account other data, including human data in other indications. For example, product efficacy in diseases of similar pathophysiology might provide supportive information for the indication being sought.

Therefore, data from appropriate studies to address each of the above bullet points would need to be provided to support the conclusion that the product is effective.

In addition, approval under this regulation will be subject to three requirements:

1. Postmarketing studies: The applicant must conduct postmarket studies, such as field studies, to verify and describe the drug's clinical benefit and to assess its safety when used as indicated when such studies are feasible and ethical (for example during or following a bioterrorism event).
2. Approval with restrictions to ensure safe use: If FDA concludes that a drug product shown to be effective under this regulation can be safely used only if distribution or use is restricted, FDA will require such postmarket restrictions as are needed to ensure safe use of the drug product.
3. Information to be provided to patients: For drug products or specific indications approved under this subpart, applicants must prepare, as part of their proposed labeling, information sheet to be provided to patients that includes the following: a disclaimer that the drug's approval was based on solely animal efficacy studies, the

indication, directions for use, foreseeable risks and benefits, and any other relevant information required by FDA at the time of approval.

FDA may withdraw approval if:

- A postmarket clinical study fails to verify clinical benefit;
- The applicant fails to perform the postmarket study with due diligence;
- Use after marketing demonstrates that postmarket restrictions are inadequate to ensure safe use of the drug product;
- The applicant fails to adhere to the postmarket restrictions applied at the time of approval under this subpart;
- The promotional materials are false or misleading; or
- Other evidence demonstrates that the drug product is not shown to be safe or effective under its conditions of use.

Animal Models of Pneumonic Plague

Pneumonic plague has been evaluated in three animal models, the mouse, Sacred Baboon and the African Green Monkey models in published literature. The Murine model and Sacred Baboon model has not been reviewed by FDA. Four natural history studies and two treatment studies (ciprofloxacin, levofloxacin) in pneumonic plague in the African Green Monkey are under review at FDA.

Studies of Pneumonic plague in mice and rats evaluated the activity of levofloxacin for prophylactic and therapeutic uses. Overall, the results demonstrated that levofloxacin dosed at 5 to 15 mg/kg/day once daily for 6 days provided complete protection in both mice and rats without any noticeable toxic effects. Levofloxacin treatment initiated early, no later than 36 hours post-challenge in mice and 42 hours post-challenge in rats had complete protection. However, when re-challenged with *Y. pestis*, levofloxacin-treated mice and rats behaved differently with the majority of mice dying and the majority of rats surviving. Levofloxacin was equally effective in neutropenic and non-neutropenic mice in the post-exposure murine model.

The development program included the following studies:

Natural History in African Green Monkey:

- Study 617 performed at Battelle Memorial Institute
- Study 875 performed at Battelle Memorial Institute
- Study performed at Lovelace Biomedical and Environmental Research Institute (LBERI)
- USAMRIID Study

The table below summarizes findings in the natural history studies of primary pneumonic plague in the African Green Monkey model.

Table 8. Summary of Findings for Primary Pneumonic Plague Natural History Studies in AGM

Parameter	Battelle Study 617	Battelle Study 875	LBERI	USAMRIID	All Natural History Studies
Aerosolized Dose of <i>Y. pestis</i> CO92 Strain	106 LD ₅₀ to 1150 LD ₅₀	24 LD ₅₀ to 88 LD ₅₀	44 LD ₅₀ to 255 LD ₅₀	9 LD ₅₀ to 57 LD ₅₀	9 to 1,150 LD ₅₀
Animals	3 males and 7 females	5 males and 5 females	5 males and 5 females	3 males and 3 females	16 males & 20 females
Mortality	100% 10 found dead	100% 10 found dead	100% (3 found dead, 7 euthanized)	67% (4/6: 1 found dead, 3 euthanized)	67 - 100%
Average Time to Death (range)	64 hours (59 to 85 hours) estimated	93 hours (66 to 139 hours)	90 hours (72 to 100 h)	112 h or 4.7 days (99.5 -125 h)	64-122 hours
Average Time to Fever Onset (range)	44 hours (39 to 49.5 hours)	54 hours (47 to 61.5 hours)	64 hours (46 to 80.5 hours)	73 hours (70 to 76 hours)	44-73 hours
Average Interval between Fever and Death (range)	28 hours (17 to 42 hours)	38 hours (16 to 57 hours)	23 hours (15 to 38 hours)	39 hours or 1.5 days (29 - 51 h)	23 - 39
Initial Bacteremia (cfu)	33 cfu to >650,000 cfu/mL	600 cfu to >7,000,000 cfu/mL	10 cfu to >200,000 cfu/mL	3 x 10 ⁶ to 9 x 10 ⁸ cfu/mL (terminal)	33 CFU/mL to 7,000,000CFU/mL
Timing of Bacteremia Onset (range)	48 to 72 hours (100% + by Day2)	48 to 72 hours (50% + by Day 2)	66 hours (43 to 94 hours)	60 hours (48 to 72 hours)	48 to 66 hours
Radiographic abnormalities	n/a	n/a	5 / 10 on Day 3	4 / 4 symptomatic animals at 80 to 83h post-exposure	5 / 10 on Day 3 4 / 4 at 80 to 83h
Symptoms	Fever, loss of appetite, respiratory distress, bloody respiratory secretions, lethargy	Fever, hunched posture, respiratory distress, bloody respiratory secretions, lethargy	Fever, loss of appetite, diarrhea, lethargy	Fever, loss of appetite, respiratory distress, lethargy	Fever, lethargy, loss of appetite, diarrhea, hunched posture, respiratory distress, bloody respiratory secretions
Labs	Neutrophilic leukocytosis, LFT, RFT abnormalities	Neutrophilic leukocytosis, LFT, RFT, coagulation abnormalities	Leukocytosis	Neutrophilic leukocytosis, RFT abnormalities	Neutrophilic leukocytosis LFT, RFT abnormalities
Pathology	hemorrhagic pneumonia	hemorrhagic pneumonia	Fibrinosuppurative hemorrhagic pneumonia	Fibrinosuppurative hemorrhagic pneumonia	Fibrinosuppurative and/or hemorrhagic pneumonia

Study FY07-070: An Efficacy Study of Intravenous Infusion of Levofloxacin in Inhalational Plague *Yersinia Pestis* Strain CO92 in Telemetered African Green Monkeys.

Overview of Study Design

Study FY07-070 was conducted at LBERI during 2008 and 2009. The study was conducted in three separate cohorts of African Green Monkeys (*Chlorocebus aethiops*). In each cohort, animals were challenged via head-only aerosol inhalation to a multiple fifty percent lethal dose (LD₅₀) of *Yersinia pestis*, strain CO92, to determine if intravenous infusion of levofloxacin would prevent death from established pneumonic plague. Established pneumonic plague was indicated by a body temperature of a mean fever > 39°C for at least one hour, which was the signal to initiate levofloxacin or placebo infusions. A total of 24 African Green monkeys (12 males and 12 females) were challenged over three days (Cohorts 1, 2 and 3) to 3-145 LD₅₀ (65 ± 47.7) to *Y. pestis*. Post-challenge, the animals were monitored via telemetry to determine the onset of defined fever. The animals were treated within six hours (3.4 ± 1.8 hours) of onset of fever with either the test article, levofloxacin IV (5 mg levofloxacin /mL5% dextrose), or control article, 5% dextrose IV, depending on predetermined treatment group. Seventeen AGMs (9 females and 8 males) were treated with test article and seven AGMs (3 females and 4 males) received control article. For each animal, the infusion of levofloxacin was administered as a “humanized” dose regimen consisting of two infusions every 24 h period in order to target plasma levels achieved in humans with a single dose of levofloxacin every 24 h.

Thus in each 24 h period levofloxacin, 8 mg/kg (high dose) or control article was administered over 30 minutes followed by a second infusion of levofloxacin [2 mg/kg (low dose)], or control article administered over 30 ± 8 minutes within 12 (± 1.0) h. Infusions continued until the death of the animal or until 20 total infusions (10 high doses and 10 low doses) had been delivered. Animals were monitored for up to 28 days post-challenge by twice daily observation and continuous monitoring of heart rate, respiration rate and temperature via implanted telemeters.

Baseline telemetry data were collected pre-challenge along with body weight, hematology, clinical chemistry, blood bacterial status, plasma levofloxacin concentration, and thoracic radiographs. For Cohorts 1 and 2, arterial blood gas, blood coagulation parameters and echocardiography were also measured. Post-challenge measurements included daily bacterial blood culture for the first 6 days, or until clearance was confirmed, and weekly thereafter, plasma levofloxacin concentration at specified intervals, and hematology and clinical chemistry parameters on Days 2 and 6. If possible chest radiographs were taken prior to euthanasia. Chest x-rays were performed pre exposure and post-exposure in Cohorts 1 and 2. A terminal necropsy was performed for surviving animal at day 28 and interim necropsies were performed on animals that died or were euthanized prior to the end of the study. Levofloxacin levels for pharmacokinetic (PK) analyses were measured throughout the study.

Following euthanasia or spontaneous death all animals were weighed, blood collected for bacterial quantitation, hematology and clinical chemistry parameters and received a gross

necropsy. Select tissues from the lungs (lesion and non-lesion), liver, spleen, kidneys and brain were collected for bacterial quantitation and histopathological examination.

Study Cohorts Cohorts 1, 2, and 3 were studied separately. Cohort 3 was added as an amendment to evaluate pharmacokinetics of levofloxacin. Double lumen central catheters were used in cohort 3 because there was a problem with bacterial contamination of blood cultures from single lumen central catheters in cohort 1.

Table 9. Study No. FY07-070 - Study Cohorts

Cohort	Study Start Date	<i>Y. pestis</i> Challenge	Control Article: 5% dextrose IV	Test Article: Levofloxacin IV
1	03/25/2008	8	3	5
2	05/02/2008	6	2	4
3	01/23/2009	10	2	8
No. of Animals		24	7	17

Materials and Methods

Test Drugs: The test article for this study was levofloxacin, Levaquin® Injection Premix in Single-Use Flexible Containers (5 mg levofloxacin/mL 5% dextrose), Ortho-McNeil, Inc. [Raitan, NJ (a subsidiary of Johnson & Johnson Companies (New Brunswick, NJ. The control was 5% Dextrose (in water) for Injection, Lot P210609. The test and control articles were prepared on the day of administration.

Challenge with *Y. pestis*: The challenge organism for this study was *Y. pestis* CO92. The challenge organism was characterized by LBERI in an ABSL-3 microbiology laboratory. All work done with *Y. pestis* CO92 strain was carried out under BSL3 conditions.

Preparation of material for aerosolization: Following centrifugation of a suspension of *Y. pestis*, the cell pellet was suspended in 1% peptone and the optical density at 600 nm (OD600) was determined. The bioaerosol sprays were prepared in Brain Heart Infusion Broth (BHIB) from the suspended centrifuged culture based on the OD600 and a previously prepared concentration/OD curve. The suspended culture was adjusted as required to achieve the target aerosol exposure level.

Study Population (Test Animals): African Green monkey (*Chlorocebus aethiops*) jacketed with implanted telemeters and intravenous catheters. Animals were conditioned to a restraint collar, poles, restraint chairs, and limb restraints. Room temperature was 18-29 °C. The animal room had 15 hours of light and nine hours of darkness per day. The animals were received from NIH c/o Alpha-Genesis, Inc. (Yemassee, SC). The animals

were approximately 3 - 8 kg and at least 2 yrs old when assigned to the study. Animals were wild-caught on the island of St. Kitts, therefore, actual ages were unknown. Animal rooms and cages were cleaned and sanitized prior to use. Animals were individually housed in stainless steel cages with wire mesh bottoms. Excreta pans under the cages, cage flooring, and room floors were cleaned daily. Monkeys were transferred to sanitized cages every two weeks, except for one protocol deviation. Drinking water was provided ad libitum. Animal rooms were lighted with fluorescent lights and maintained on a 15-hour light/9-hour dark cycle beginning at least one week prior to exposure and during the assay period, except as required during designated procedures, e.g., infusions.

Implantation of study monitoring devices: Animals were surgically manipulated to install telemetry monitoring devices, then intravenous catheters, and then moved into the ABSL-3 at least one week prior to challenge with *Y. pestis*. All animals had T30F telemeters (DISS, Inc.) implanted. T30F telemeters provided continuous monitoring of body temperature, intrathoracic pressure, respiratory rate, heart rate, and basic electrocardiographic signal traces detecting major arrhythmias. The telemeters were placed on the left abdominal wall by experienced surgical personnel, monitored post-operatively in standard housing to permit the wound healing to complete and the natural fibrotic reactions to fix the telemetry sensors in place. All animals had venous access catheters [Broviac (Cohorts 1 and 2) or Hickman [dual-port (Cohort 3)]] inserted in the right femoral vein. The catheter was tunneled through the right flank and back, emerging through the skin of the upper mid-back. The exit site and catheter were protected by a jacket. No study animals received systemic antibiotics within 28 days prior to aerosol exposure with *Y. pestis* strain CO92 or topical Mupiricin ointment within 14 days of aerosol exposure. Animals were identified by tattoo, jacket number, telemetry frequency, and cage card. African Green monkeys, jacketed, with implanted telemeters, intravenous catheters in the femoral vein for blood withdrawal and infusion of test and control articles and trained to poles and collars, chairs and limb restraints were assigned to the study.

Aerosol - *Y. pestis*: Nebulizer solutions of *Y. pestis* strain CO92 were formulated on Study Day 0 for each Cohort. The target particle size was 1 to 3 μm . All study animals were exposed to aerosolized *Y. pestis* at an approximate target dose of 100 LD₅₀ (actual range was 3 – 145 LD₅₀ on Day 0).

Levofloxacin Dose: Animals were monitored by telemetry for increase in temperature. Test animals were infused with levofloxacin, or an equal volume of control article in control animals, within 6 hours of the onset of fever. In each individual animal, an infusion of test article [levofloxacin, 8 mg/kg (high dose)] or control article was administered over 30 ± 5 minutes within every 24 ± 0.5 h. To mimic the human pharmacokinetics of levofloxacin, a second infusion of levofloxacin [2 mg/kg (low dose)], or vehicle, was administered over 30 ± 5 minutes within 12 h after the 24 h (i.e., high dose) infusion. Infusions continued until the death of the animal or until 20 total infusions (10 high doses and 10 low doses) had been delivered.

Telemetry: Animals were monitored by telemetry for temperature, respiratory rate, and heart rate. Test animals were infused with levofloxacin, or an equal volume of control article in control animals, within 6 hours of the onset of fever. In each individual animal, an infusion of test article [levofloxacin, 8 mg/kg (high dose)] or control article was administered over 30 ± 5 minutes within every 24 ± 0.5 h. To mimic the human pharmacokinetics of levofloxacin, a second infusion of levofloxacin [2 mg/kg (low dose)], or vehicle, was administered over 30 ± 5 minutes within 12 h after the 24 h (i.e., high dose) infusion. Infusions continued until the death of the animal or until 20 total infusions (10 high doses and 10 low doses) had been delivered.

Randomization: Animals were assorted into test groups using a validated computerized data acquisition system (Path-Tox 4.2.2; Xybion, Cedar Knolls, NJ) based on bodyweights. Animals were randomized into housing placement and exposure order using Microsoft Excel's® random number generator.

Clinical Reviewer's Comment: *A study of aerosolized Y. pestis in telemetered AGMs (NIAID Study No. D13-01, Study No, FY06-126 conducted at LBERI) determined the optimal timing for initiation of intravenous (IV) antibiotic therapy, triggered by the onset of fever > 39°C as the first sign of systemic disease.*

RESULTS

Study Population

Twenty-six AGM were randomized to the control (n=7) group or the levofloxacin (n=19) treatment group. Animal X779 was removed from the study post-randomization for a protocol deviation because it received a dose of IV levofloxacin prior to development of a fever. Animal X717 was removed from the study post-randomization due to illness - it did not receive a challenge dose of *Y. pestis* and was not treated with levofloxacin. Cohorts 1, 2, and 3 were studied separately. Cohort 1, 2, and 3 contained 8, 6, and 10 animals, respectively and included 2 or 3 control animals per cohort.

Figure 1. Study Population

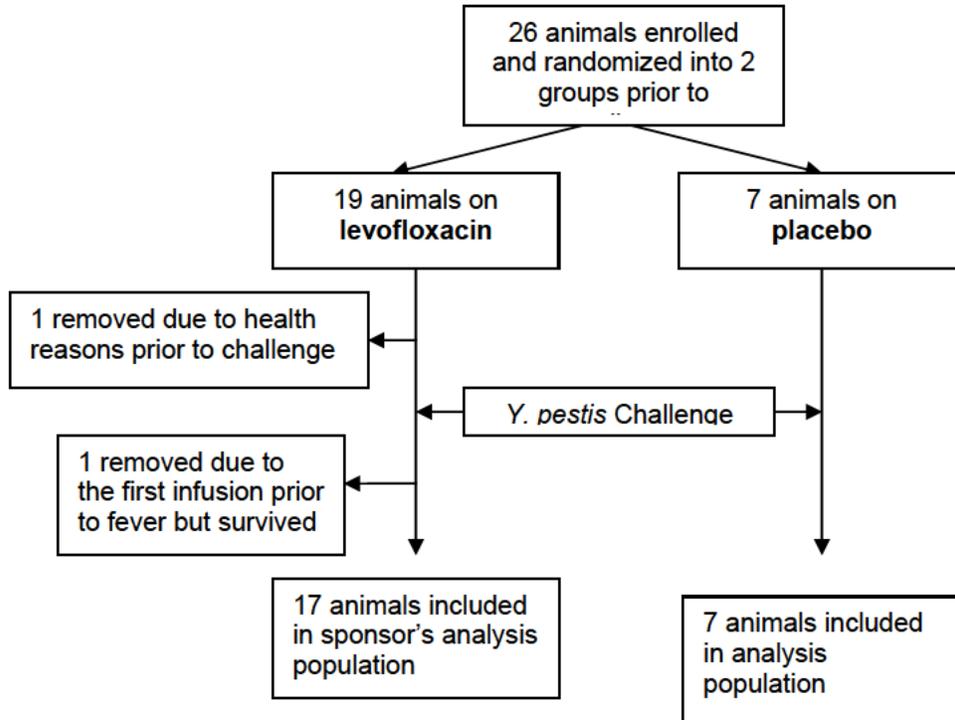


Table 10. Animals in the Control and Levofloxacin-Treatment Group

Animal ID Controls, n=7	Levofloxacin Group, n=17
X702	X663
X773	X662
X762	X648
U193	X437
U734	X523
X888	X732
Y283	X419
	X771
	X761
	Y160
	Y217
	Y226
	Y295
	Y275
	Y276
	Y293
	Y301

Analysis performed using JReview 9.2

Baseline Characteristics of African Green Monkeys

Twenty-four African Green Monkeys remained in the study, 12 males and 12 females. All animals were weighed several times during the weeks of screening process. The following listed weights were taken immediately prior to exposure to aerosolized *Y. pestis*. The mean body weight was 4.9 kg (3.7 - 7.1 kg) taken immediately prior to exposure to aerosolized *Y. pestis*.

Clinical Reviewer's Comment: These weights were chosen by the reviewer as the most relevant baseline from which to assess for on-going weight loss during the study. Animals had body weights measured several times prior to the study as they acclimatized to the laboratory environment and all animals lost some weight during this period.

Table 11. Study FY07-070: Baseline Characteristics of African Green Monkeys

Animal ID	Male/Female (count)	Baseline weight (Kg)
U193	F	4.22
X419	F	4.03
X437	M	5.17
X523	M	5.68
X648	F	4.54
X662	F	3.73
X663	F	3.82
X702	F	4.98
X732	F	3.98
X734	M	5.30
X761	M	5.50
X762	M	4.93
X771	M	5.27
X773	M	7.15
X888	F	3.89
Y160	F	3.89
Y217	F	3.98
Y226	F	3.93
Y275	M	6.42
Y276	M	6.52
Y283	M	6.64
Y293	M	6.01
Y295	F	3.84
Y301	M	3.84
Total Subjects =24	12 M, 12 F = 24	Mean Weight: 4.9 Kg

Analysis performed using JReview 9.2

Inhalation Exposure to Aerosolized *Y. pestis*

Each cohort of AGM was exposed to *Y. pestis* CO92 during a single study day. The mean calculated LD₅₀ was 74 ± 31.0 LD₅₀, 124 ± 10.5 LD₅₀, and 22 ± 23.1 LD₅₀ for cohort 1, 2, and 3, respectively, Table 13. Among the three cohorts, Cohort 3 had the lowest mean calculated LD₅₀ (± SD) = 22 ± 23 LD₅₀. Six of the animals in the levofloxacin-treatment group in Cohort 3 received challenge doses ranging from 3 to 12 LD₅₀ which is at least four fold lower than the two control animals within the cohort. The two control animals received 40 LD₅₀ and 47 LD₅₀, respectively, and both animals died from pneumonic plague. The calculated inhaled doses and calculated LD₅₀ inhaled doses for each monkey is summarized in the table below.

Table 12. Challenge Doses of *Y. pestis* LD₅₀ in Control and Levofloxacin-treated AGM

Cohort	Animal ID	LD ₅₀ equivalents	LD ₅₀ Mean ± SD
1	X437	40	
	X523	75	
	X648	57	
	X662	81	
	X663	66	
	X702	56	
	X762	76	
	X773	143	74 ± 31.0 LD ₅₀
2	U193	121	
	X419	120	
	X732	124	
	X734	145	
	X761	118	
	X771	118	124 ± 10.5 LD ₅₀
3	X888	44	
	Y160	6	
	Y217	38	
	Y226	12	
	Y275	4	
	Y276	3	
	Y283	47	
	Y293	62	
	Y295	3	
	Y301	3	22 ± 23.1 LD ₅₀

Analysis performed using JReview 9.2

Comparison of Plague in Control AGM in Study FY07-070 to AGM in the Four Natural History Studies

A comparison of the control animals in the efficacy study, Study FY07-070, to animals exposed to *Y. pestis* in the four natural history studies is summarized in Table 13. The target challenge dose was 100 LD₅₀ ± 50 LD₅₀ in the natural history studies and this range was achieved in the current efficacy study.

Animals in the control group were similar to the natural history animals with regard to mortality rates, time to death, timing of fever onset, interval between fever and death, onset of bacteremia, and histopathologic findings in the lung.

Table 13. Comparison of Control Group in Study FY07-070 to Natural History Studies

Parameter	Controls Study No. FY07-070	Natural History Study
Aerosolized Dose of <i>Y. pestis</i> CO92	56 – 145 LD ₅₀	20 – 1150 LD ₅₀
Mortality	100% (7/7)	100% (34/34)*
Time to Death	97 – 133 h	66 – 139 h
Fever Onset	58 – 93 h	39 – 90.5 h
Interval between fever and death	26 – 48 h	29 – 51 h
Bacteremia Onset	49 – 93 h (7/7)	48 – 94 h (34/34)
Histopathology – Lung	Fibrino-suppurative hemorrhagic pneumonia	Fibrino-suppurative hemorrhagic pneumonia

*Two animals that received < 20 LD₅₀ survived and showed no symptoms of clinical disease

Clinical Reviewer's Comment: Lethality due to pneumonic plague has been demonstrated in the AGM at > 20 LD₅₀ inhaled doses. Two animals that received < 20 LD₅₀ survived in the natural history studies. Upon evaluation of the data from four AGM natural history studies, the review team noted that despite significant variability in the actual challenge dose received (9 to >1000 LD₅₀), exposures > 20 LD₅₀ induced signs and symptoms of pneumonic plague and are uniformly fatal.

Challenge Dose of Aerosolized *Y. pestis*

All animals in Cohort 1 and Cohort 2 received challenge doses > 20 LD₅₀ of *Y. pestis*. Six animals in the levofloxacin-treatment group in Cohort 3 received challenge doses of 3 - 12 LD₅₀. All animals developed a fever and all became bacteremic post-challenge. Five of these six animals survived and one animal, Y160, was euthanized because of a pathological process in the stomach which caused vomiting and the animal was not able to keep food down. The two control animals in Cohort 3 received challenge doses of 47 LD₅₀ and 44 LD₅₀ respectively and both died of pneumonic plague.

The characteristics of the six animals with low challenge doses (LD₅₀) are summarized in Table 14.

Table 14. Characteristics of AGM that received a challenge dose < 20 LD₅₀ of *Y. pestis*

Cohort 3	Treatment Group	Challenge Dose <i>Y. pestis</i>	Post exposure Fever > 39 °C	Blood Culture <i>Y. pestis</i>	Chest X-ray	Tissue	Survived
Animal ID		LD ₅₀ equivalents		+ / -	Follow up	Lung tissue culture	Survival
Y160	Levofloxacin	6	Yes	+	N/D	contaminant	Died Day 9
Y226	Levofloxacin	12	Yes	+	N/D	neg	Yes
Y275	Levofloxacin	4	Yes	+	N/D	neg	Yes
Y276	Levofloxacin	3	Yes	+	N/D	neg	Yes
Y295	Levofloxacin	3	Yes	+	N/D	neg	Yes
Y301	Levofloxacin	3	Yes	+	N/D	neg	Yes

N/D: follow up chest x-ray not done; + : positive blood culture; - : negative blood culture.
 Analysis performed using JReview 9.2

Clinical Comment: All these animals were bacteremic with *Y. pestis*, therefore it is likely that, even with the relatively low challenge doses of *Y. pestis*, they would have succumbed to plague if they had not been treated with IV levofloxacin.

Levofloxacin Dosing Regimen

Levofloxacin was infused at 8 mg/kg/day body weight, followed by 2 mg/kg administered approximately 12 h later, calculated using the body weight recorded at the time of aerosol exposure. One animal (Y295) received 6.5 mg of levofloxacin at one low dose infusion time point, which did not impact on study results as it had negative blood culture following the first infusion.

Onset of Fever

Per the protocol, the trigger to start treatment with levofloxacin IV or placebo was the onset of fever defined as a mean body temperature > 39°C for at least one hour recorded by telemetry. All animals developed a fever > 39 °C for more than one hour within the first seven days post exposure to aerosolized *Y. pestis*. The majority of animals developed fever within 3 days post-exposure. Two animals developed a fever on Day 4, two on Day 5 and one on Day 7 post-exposure, X702 and Y275 developed fever within 93 to 97 hours (day 4). Three outliers, X437, Y276 and Y295 which developed fever on day 5 or day 7 (124 to 166 hours) post-challenge. All levofloxacin-treated animals were febrile at the start of treatment except one animal, X779, received a dose of levofloxacin prior to development of fever.

Time Interval between Aerosol Challenge to Onset of Fever and Start of Treatment

The time interval from exposure to aerosolized *Y. pestis* and onset of fever ($> 39\text{ }^{\circ}\text{C}$ for 1 hour) ranged from 53 to 166 hours. The LD_{50} exposures, though low, in some animals did not appear to account for the delay in the development of fever as two animals with low levels of exposure i.e. Y160 (6LD_{50}) and Y301 (3LD_{50}) developed a fever at 65 and 67 hours post-challenge, respectively.

Infusion of placebo or levofloxacin

The infusions of placebo or levofloxacin were started within 6 hours of fever for all animals in the study. A summary of interval time (hours) from challenge to development of fever and from fever ($> 39\text{ }^{\circ}\text{C} \times 1\text{ hour}$) to the first infusion is shown in Table 15.

Table 15. Fever Onset post-exposure to *Y. pestis* in Control and Levofloxacin-treated animals by study hour

	Aerosol exposure	Aerosol challenge	Fever start	Interval in Hours	Infusion Start - Study Hour
Animal ID*	LD₅₀ equivalents	study hour	study hour	Aerosol to fever	(interval in hrs fever to Rx)
U193	121	12	70	58	72 (2)
X702	56	13	106	93	107(1)
X734	145	10	68	58	71(3)
X762	76	11	85	74	86(1)
X773	143	11	76	65	80 (4)
X888	44	11	84	73	89 (5)
Y283	47	10	78	68	83 (5)
X419	120	13	74	63	80 (6)
X437	40	9	133	124	135(2)
X523	75	10	63	53	68 (5)
X648	57	13	84	73	85 (1)
X662	81	12	80	68	81 (1)
X663	66	12	72	60	73 (1)
X732	124	10	81	71	83 (2)
X761	118	12	82	70	84 (2)
X771	118	11	86	75	87 (1)
Y160	6	13	78	65	84 (6)
Y217	38	11	83	72	88 (5)
Y226	12	14	80	66	84 (4)
Y275	4	14	106	92	112 (6)
Y276	3	12	178	166	182 (4)
Y293	62	10	83	73	88 (5)
Y295	3	12	137	125	141(4)
Y301	3	13	80	67	85 (1)

Source: Analysis using JReview 9.2

*Control animals ID are in **bold** type

Death

Death occurred within 85 to 122 hours in control animals post-challenge with aerosolized *Y. pestis*, Table 16. The time interval from fever to death was 26 to 48 hours for control animals, similar to the natural history studies. Animal Y160 in the levofloxacin-treatment group died on Day 9 or 204 hours post-challenge with *Y. pestis* and 139 hours after onset of fever; this animal is described in more detail below.

Table 16. Time from aerosol challenge to onset of fever and death

			Aerosol Challenge	Time interval Aerosol to fever	Outcome: Live/Death /Euthanasia	Time Interval Aerosol to Death	Time Interval Fever to death
Cohort	Animal ID	Treatment	Study start hour	Hours	Study Hour	Hours	Hours
1	X437	Levofloxacin	9	124	Live (684)	-	-
	X523	Levofloxacin	10	53	Live (680)	-	-
	X648	Levofloxacin	13	71	Live (683)	-	-
	X662	Levofloxacin	12	68	Live (682)	-	-
	X663	Levofloxacin	12	60	Live (682)	-	-
	X702	Control	13	93	132	119	26
	X762	Control	11	74	133	122	48
	X773	Control	11	65	124	113	48
	2	U193	Control	12	58	97	85
X419		Levofloxacin	13	61	Live (682)	-	-
X732		Levofloxacin	10	71	Live (679)	-	-
X734		Control	10	58	96	86	28
X761		Levofloxacin	12	70	Live (681)	-	-
X771		Levofloxacin	11	75	Live (680)	-	-
3	X888	Control	11	73	120	109	36
	Y160	Levofloxacin	13	65	217	204	139
	Y217	Levofloxacin	11	72	Live (683)	-	-
	Y226	Levofloxacin	14	66	Live (684)	-	-
	Y275	Levofloxacin	14	92	Live (681)	-	-
	Y276	Levofloxacin	12	166	Live (682)	-	-
	Y283	Control	10	68	115	105	37
	Y293	Levofloxacin	10	73	Live (680)	-	-
	Y295	Levofloxacin	12	125	Live (684)	-	-
	Y301	Levofloxacin	13	67	Live (682)	-	-

Analysis performed using JReview 9.2

Efficacy Analyses

The primary efficacy endpoint was survival at end of study, Day 28. The primary analysis population was the intent-to-treat (ITT) population. Efficacy analyses were performed on the following populations:

Intention-to-treat (ITT) Population: All AGM that were randomized and received an aerosol challenge with *Y. pestis* were included in the analysis. The ITT analysis was based on the treatment group planned at randomization. Animals that received the aerosol challenge, but died before or during treatment with placebo or active drug, or had a protocol deviation were included in this population as treatment failures.

Animals that received a challenge dose > 20 LD₅₀: A cut value of >20 LD₅₀ was chosen because animals that were untreated and received ≤20 LD₅₀ *Y. pestis* survived in a natural history study of pneumonic plague in the AGM model.

Animals that were bacteremic (Y. pestis) at treatment: Animals that were bacteremic (evidenced by a positive blood culture for *Y. pestis*) at the time of therapeutic intervention.

Animals that had radiographic evidence of pneumonia: Animals that had evidence of pulmonary infiltrates on chest radiograph consistent with pneumonia.

Survival in the ITT population: Levofloxacin-Treated versus Placebo-Treated Animals

None of the control animals survived, all were dead or euthanized by Day 5. Three of the control animals (U193, X762, X773) were euthanized moribund and four animals died spontaneously. A total of 16/17 (94%) levofloxacin-treated animals and none of the control animals survived to the end of the study at Day 28.

Animal, X717 in the levofloxacin treatment group was removed from the study post-randomization but it did not receive a challenge dose of *Y. pestis*, was not treated with levofloxacin, and was not included in the ITT analysis.

In the levofloxacin-treated group, one animal, X779, was removed from the study post-exposure to *Y. pestis* for a protocol deviation - it received a first dose of levofloxacin prior to development of fever. The applicant informed the FDA reviewers that the AGM was followed off protocol and it received no additional antibiotic therapy and it survived to the end of the study period.

Additional analyses were conducted with both animals included as non survivors as well as X717 included as non-survivor and X779 included as survivor; the survival analyses were statistically significant, see footnote, Table 17.

Table 17. Survival in ITT Population

	Levofloxacin*	Placebo
Survival (%)	16/17 (94%)	0/7
95% CI	55.5%, 99.9%	

P value	<0.0001	
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*If X717 included as non-survivor and X779 included as survivor, 95% CI [42.1%, 98.7%], p-value <0.0001.

*If both included as failures, 95% CI [42.1%, 96.6%], p-value 0.0002.

Exact 95% confidence interval for difference in survival proportion was calculated. Fisher's exact test (two-sided).

Animal Y160 - euthanized DAY 9

Animal Y160 was euthanized moribund for severe vomiting due to an ill-defined stomach problem on Day 9, and was considered a treatment failure for this analysis. Beginning on Day 7, the animal appeared to be vomiting red material that looked like tissue, this observation continued through Day 8 and 9, and the animal was unable to retain food. The animal was euthanized on Day 9 with the consensus of a staff veterinarian and the Principal Investigator.

The following is a summary of the clinical observations for the animal:

Day 1, 2, 3, 5: Normal behavior in cage;

Day 1 to 7: Reduced appetite, eating enrichment;

Day 4,6,7,8 in AM: Hunched and hypoactive; Day 0-7: normal appearance in PM-;

Day 6 & 7: Dry cough;

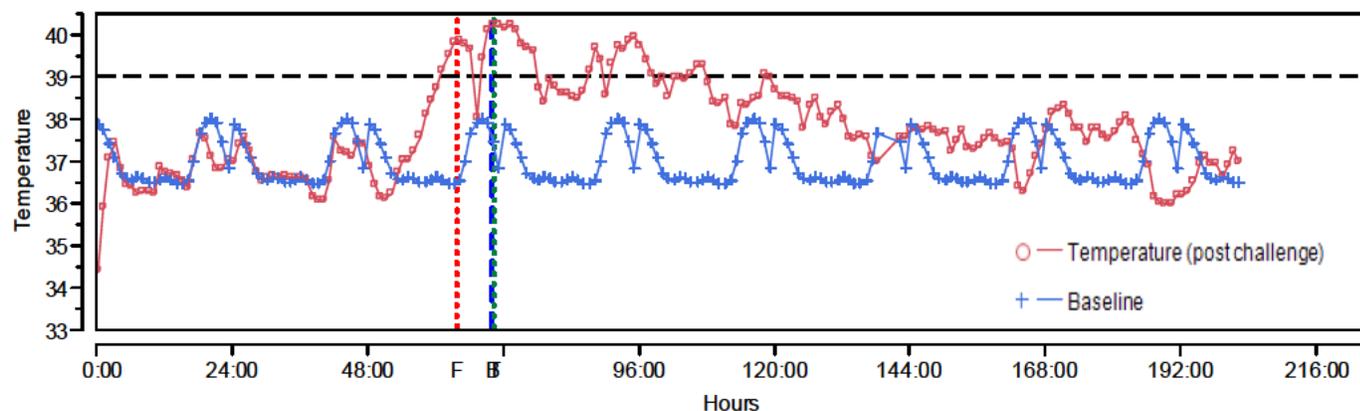
Day 7: Vomiting;

Day 8: Vomiting worsening; subdued when stimulated.

The animal appeared to be responding to levofloxacin therapy because its body temperature trended toward baseline from Day 6 to Day 9. Additionally, it had a positive blood culture on Day 3 and all subsequent daily blood cultures (Day 4 – 7) were negative. The animal had a normal chest radiograph prior to challenge and a chest x-ray performed on the day of euthanasia revealed bilateral interstitial infiltrates (R>L), described as "moderate" by the radiologist.

Tissue samples collected at necropsy were negative for *Y. pestis*. Histopathological examination of the stomach revealed necrosis of the gastric epithelium. The animal's body temperature pattern from Day 0 - 9 is outlined in the following graph, Fig 2.

Figure 2. Animal Y160 - Body Temperature Pattern, Day 0 - 9



F=fever onset; B= Bacteremia; T= treatment with IV levofloxacin

Analysis performed using JMP 9.0.2

Best Available Copy

This death did not appear to be related to *Y. pestis* challenge but the underlying cause of the stomach problem that led to its moribund state is uncertain.

Sensitivity Analyses of Survival in Sub-populations of AGM

In addition to the primary analysis of survival in the ITT population, survival was also assessed in animal who had challenge doses > 20LD₅₀, bacteremic animals, and animals with radiologic evidence of pneumonia.

Animals that received a challenge dose > 20 LD₅₀ *Y. pestis*

All animals in Cohort 1 and Cohort 2 received challenge doses > 20 LD₅₀ of *Y. pestis*. The cut-off of 20 LD₅₀ was chosen based on the natural history studies, which demonstrated that animals that succumbed to pneumonic plague had a challenge dose > 20 LD₅₀ of *Y. pestis* CO92 strain. Six animals in the levofloxacin-treated group in Cohort 3 received a challenge dose of 3 to 12 LD₅₀ and these animals are excluded from the following analysis. The two control animals in Cohort 3 received challenge doses of 47 LD₅₀ and 48 LD₅₀, respectively, and both died of pneumonic plague.

Table 18. Survival in Animals with > 20 LD₅₀

	Levofloxacin	Placebo
Survival (%)	11/11 (100%)	0/7
95% CI	58.9%, 100%	
P value	<0.0001	

Six levofloxacin-treated animals that received a challenge dose of < 20 LD₅₀ are excluded from analysis however all became bacteremic. Five of these animals survived to the end of the study, Day 28

Animals that were Bacteremic at Start of Treatment

Five levofloxacin-treated animals (one in cohort 1; three in cohort 2; one in cohort 3) did not have positive blood cultures at the start of treatment and are excluded from the following analysis. The six animals that received a challenge dose < 20 LD₅₀ were bacteremic and are included in this analysis. Survival was 11/12 (92%) among levofloxacin-treated animals and 0% in control animals at Day 28.

Table 19. Survival in Animals with Bacteremia at Start of Treatment

	Levofloxacin	Placebo
Survival (%)	11/12 (91.7%)*	0/5†
95% CI	28.0%, 99.8%	
P value	<0.001	

*One levofloxacin-treated animal (#Y293) had 1 colony of *Y. pestis* isolated from a pre-infusion blood sample on a qualitative culture plate on Day 3, all other catheter blood plates at this time point were contaminated with colonies inconsistent with *Y. pestis*. If Animal Y293 was included in the analysis, the survival is 92% (12/13), 95% CI (28.3%, 99.8%), P value 0.0007

†Two control animals (X773 and X888) were not bacteremic prior to first infusion. They became bacteremic the day after the first infusion.

Clinical Reviewer's Comment: Levofloxacin demonstrated efficacy (92% survival in levofloxacin-treated animals versus 0% in placebo arm) for the treatment of AGM with septicemic plague.

Animals with Radiologic Evidence of Pulmonary Infiltrates

All animals in Cohorts 1 and 2 that had chest radiographs performed post-exposure to *Y. pestis* had evidence of pulmonary infiltrates. Animals in Cohort 3 had baseline chest radiographs only and were excluded from the analysis. Two of five animals in the control group in cohorts 1 & 2 died or were euthanized before chest x-rays could be performed. The survival rate in the levofloxacin-treated animals that had pulmonary infiltrates was 100%.

Table 20. Animals with Pulmonary Infiltrates on Chest Radiograph (cohorts 1 and 2)

	Levofloxacin	Placebo
Survival (%)	9/9 (100%)	0/3
95% CI	29.0%, 100%	
P value	0.005	

Clinical Reviewer's comment: *In all subpopulations that were evaluated, the proportion of survivors in levofloxacin-treated group was significantly greater than the proportion of survivors in the placebo group.*

Narrative - Animal X779

Animal X779, though it was removed from the study at an early stage, deserves further scrutiny. It received a challenge dose of 83 LD₅₀ *Y. pestis* and developed a fever >39°C for 1 hour on Day 3 post challenge or 7.5 h after the first dose of IV levofloxacin. On May 5, 2008 (3 days post challenge) it received a single dose of 8 mg/kg levofloxacin IV. On May 11 (9 days post challenge) it received 0.5 mL of a 6.29 mL (5 mg/kg) dose of levofloxacin. Blood culture samples taken on Day 2, Day 3, Day 4, Day 14, Day 21, and Day 28 post-challenge were negative for *Y. pestis*. Daily clinical observations showed signs indicative of illness including reduced appetite /anorexia, rough hair coat appearance and hunched posture primarily during days 3-10 post-challenge. It had an increased respiratory rate and heart rate at the same time of fever onset. A chest radiograph on Day 0 (prior to challenge) was normal and no additional chest radiographs were performed. It was euthanized at the end of the study period and no histopathology reports were submitted for review.

Clinical Reviewer's comment: *Clinical observations of animal X779 indicated that it was ill from pneumonic plague. The survival of this animal following only one full dose of IV levofloxacin is unusual and studies of one dose Rx with levofloxacin in AGM have not been done. However, the survival of this animal indicates that in some AGM one dose of levofloxacin may be enough to treat pneumonic plague. Animals in the study were not evaluated for pre-existing antibodies to *Yersinia* spp., therefore it is not known if the animal had some immunity to plague.*

Duration of Fever

High fevers of $\geq 39^{\circ}\text{C}$ resolved in the levofloxacin-treatment group on Day 3 to 4 post initiation of IV levofloxacin and elevations of body temperature above baseline diurnal range continued up to Day 14. The seven control animals did not return to their baseline body temperature diurnal range and had elevated respiratory rates and heart rates prior to their death/euthanasia.

Table 21. Resolution of High Fever

LEVOFLOXACIN	Fever Start	Infusion start	Resolution of high fever ($>39^{\circ}\text{C}$)
Animal ID	Study Day	Study Day	Study Day
X419	3	3	6
X437	5	5	8
X523	2	2	5
X648	3	3	6
X662	3	3	6
X663	3	3	6
X732	3	3	6
X761	3	3	6
X771	3	3	6
Y160	3	3	6
Y217	3	3	9
Y226	3	3	6
Y275	4	4	7
Y276	7	7	11
Y293	3	3	6
Y295	5	5	8
Y301	3	3	5

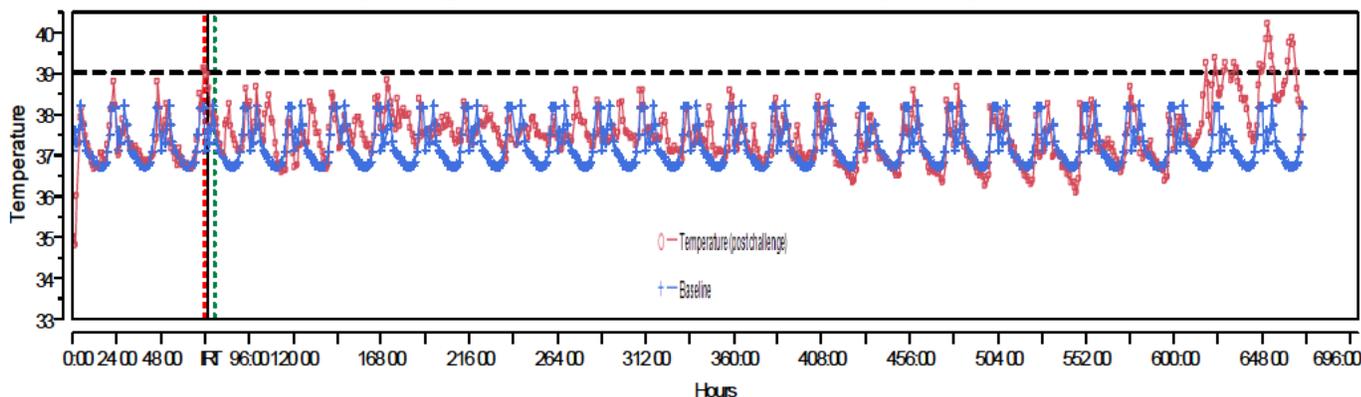
Analysis performed using JReview 9.2

Animal Y293

One animal, Y293 (levofloxacin group, cohort 3), developed a fever $> 39^{\circ}\text{C}$ in the final days of the study. It had resolution of high fever on Day 4 and returned to its baseline body temperature range by \sim Day 14. It developed a fever $>39^{\circ}\text{C}$ on Day 26 through Day 28 (terminal). The animal had received a challenge dose 62 LD₅₀ and blood cultures were negative for *Y. pestis* during the study. One colony of *Y. pestis* was isolated from a pre-infusion blood sample on a qualitative culture plate on Day 3, all other catheter blood plates at this time point grew colonies inconsistent with *Y. pestis*. The animal appeared ill and hunched over for approximately 11 days during the study on Days 5 - 9, 11, 13 - 15, 19, 21. A blood culture at the terminal blood draw had bacteria >300 CFU, not *Y. pestis*, possibly related to a contaminated IV catheter. The animal did not have a chest radiograph. Tissue cultures of spleen, liver and TBLN had bacterial

contamination, not *Y. pestis*.; however, blood and tissue cultures were negative for *Y. pestis*. Pulmonary histopathology was consistent with resolving pneumonia. The animal was euthanized therefore no follow-up is available.

Animal Y293: Body Temperature Pattern over the 28-Day Study Period



Analysis performed using JMP 9.0

Respiratory Rates and Heart Rates

Increases in respiratory rates appeared after the increase in body temperature and was followed by an increased heart rate in all animals. A statistically significant increase in respiratory rate consistently began on the evening of study Day 2 for the control and levofloxacin group animals. By Day 3, the mean increase in the control group was greater than 10 breaths/minute and rapidly increased over 20; for the levofloxacin-treated group the mean increase was 10 breaths/min. The resolution of elevated respiratory rate back to baseline rates tended to occur after resolution of significant fever in the levofloxacin-treated group.

A statistically significant increase in heart rate consistently began on the evening of Day 2 for the control group and the levofloxacin group. Increased heart rates continued throughout the study for some animals in the control group and levofloxacin-treated group.

Weight Loss

Weight loss occurred in control and levofloxacin-treated animals during the pre-study period and during the study from Day 0 to Day of death/euthanasia. The mean weight loss during the pre-study period in the control group and the animals that were to receive levofloxacin was 0.14 kg and 0.12 kg, respectively. The mean weight loss in control and levofloxacin-treated animals during the study from day 0 to day of death/euthanasia was 0.13 kg and 0.04 kg, respectively. The weight loss was statistically significant in the control group only.

Clinical Observations

The main adverse clinical signs noted were reduced activity, changes in posture, inappetence and changes in stool. Other observations noted were reduced grooming, respiratory changes, neurological signs, and nasal/ocular discharge.

The most common clinical observations included reduced activity, poor appetite, and changes in posture. All animals in the control (n=7) and levofloxacin group (n=14) had a reduced appetite or

were anorexic. All animals had reduced activity during the study and the majority of levofloxacin-treated animals (14) and the control group (7) appeared ill and hunched-over for several days during the study period.

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Table 22. Summary of Clinical Observations

Summary of Incidence of Clinical Observations

Cohort: Treatment:	Cohort 1				Cohort 2				Cohort 3				All			
	Control		Levo		Control		Levo		Control		Levo		Control		Levo	
	a ^a	b ^b														
Number of Animals in Group:	3		5		2		4		2		8		7		17	
All Observations Normal	3	1.3	5	13.2	2	1.5	4	6.0	2	1.0	7	9.9	7	1.3	16	9.7
Adverse Observations																
Respiration																
Dry Cough	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	4	0.6	0	0.0	5	0.3
Labored	1	0.3	0	0.0	1	0.5	0	0.0	0	0.0	2	0.4	2	0.3	2	0.1
Neurology																
Tremors	1	0.3	0	0.0	0	0.0	0	0.0	0	0.0	1	0.1	1	0.1	1	0.0
Lack of Coordination	1	0.3	0	0.0	0	0.0	0	0.0	0	0.0	1	0.1	1	0.1	1	0.0
Appetite																
Reduced / Anorexic	3	3.7	5	13.4	2	4.0	4	17.8	2	2.5	8	8.8	7	3.4	17	13.3
Appearance																
Hunched	3	3.0	2	0.8	1	0.5	4	5.8	2	0.5	8	12.4	6	1.3	14	6.3
Prostrate	1	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.1	0	0.0
Nasal Discharge	2	1.7	0	0.0	0	0.0	1	0.3	0	0.0	0	0.0	2	0.6	1	0.1
Ocular Discharge	0	0.0	0	0.0	1	0.5	0	0.0	0	0.0	1	0.1	1	0.2	1	0.0
Unkempt/Rough Hair Coat																
Thin	0	0.0	0	0.0	1	0.5	3	3.8	1	0.5	4	0.9	2	0.3	7	1.5
Abrasion / Laceration	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.3	0	0.0	1	0.1
Activity																
Reduced	3	2.7	4	3.8	2	1.0	4	15.3	2	1.5	8	3.5	7	1.7	16	7.5
Subdued when Stimulated	2	1.3	0	0.0	1	0.5	2	0.5	1	0.5	1	0.1	4	0.8	3	0.2
Hypoactive	1	0.3	0	0.0	0	0.0	0	0.0	2	2.0	8	10.1	3	0.8	8	3.4

^a a = Number of animals affected
^b b = Mean number of animal days with clinical sign = total number of days observation noted ÷ total number of animals in group (Maximum Number of Days = 29)

Clinical Review
 Elizabeth O'Shaughnessy, M.D.
 NDA20-634, NDA21-721, NDA20-635
 Levofloxacin, LEVAQUIN®

Summary of Incidence of Clinical Observations

Cohort:	Cohort 1		Cohort 2		Cohort 3		All									
	Control	Levo														
Treatment:	a ^a	b ^b														
Number of Animals in Group:	3	5	2	4	2	8	7	17								
Adverse Observations																
Gastrointestinal / Urogenital																
Stool - Soft	2	1.0	1	0.4	0	0.0	3	2.7	1	0.5	7	4.0	3	0.5	11	2.4
Stool - Liquid	1	0.7	0	0.0	0	0.0	1	0.3	0	0.0	6	1.0	1	0.2	7	0.4
Stool - Hard	3	1.7	5	3.6	2	0.4	4	5.7	2	1.5	8	5.5	7	1.2	17	4.9
Stool - Scant/None	0	0.0	0	0.0	0	0.0	0	0.0	2	2.0	8	6.3	2	0.7	8	2.1
Minimal Urine Output	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	5	2.9	0	0.0	5	1.0
Survival																
Natural/Spontaneous death	1	0.3	0	0.0	1	0.5	0	0.0	2	1.0	0	0.0	4	0.6	0	0.0
Moribund Euthanasia	2	0.7	0	0.0	1	0.5	0	0.0	0	0.0	1	0.1	3	0.4	1	0.0
Scheduled Euthanasia	0	0.0	5	1.0	0	0.0	4	1.0	0	0.0	7	0.9	0	0.0	16	1.0
^a a = Number of animals affected ^b b = Mean number of animal days with clinical sign = total number of days observation noted ÷ total number of animals in group (Maximum Number of Days = 29)																

Source: Study Report, Study No. FY07-070

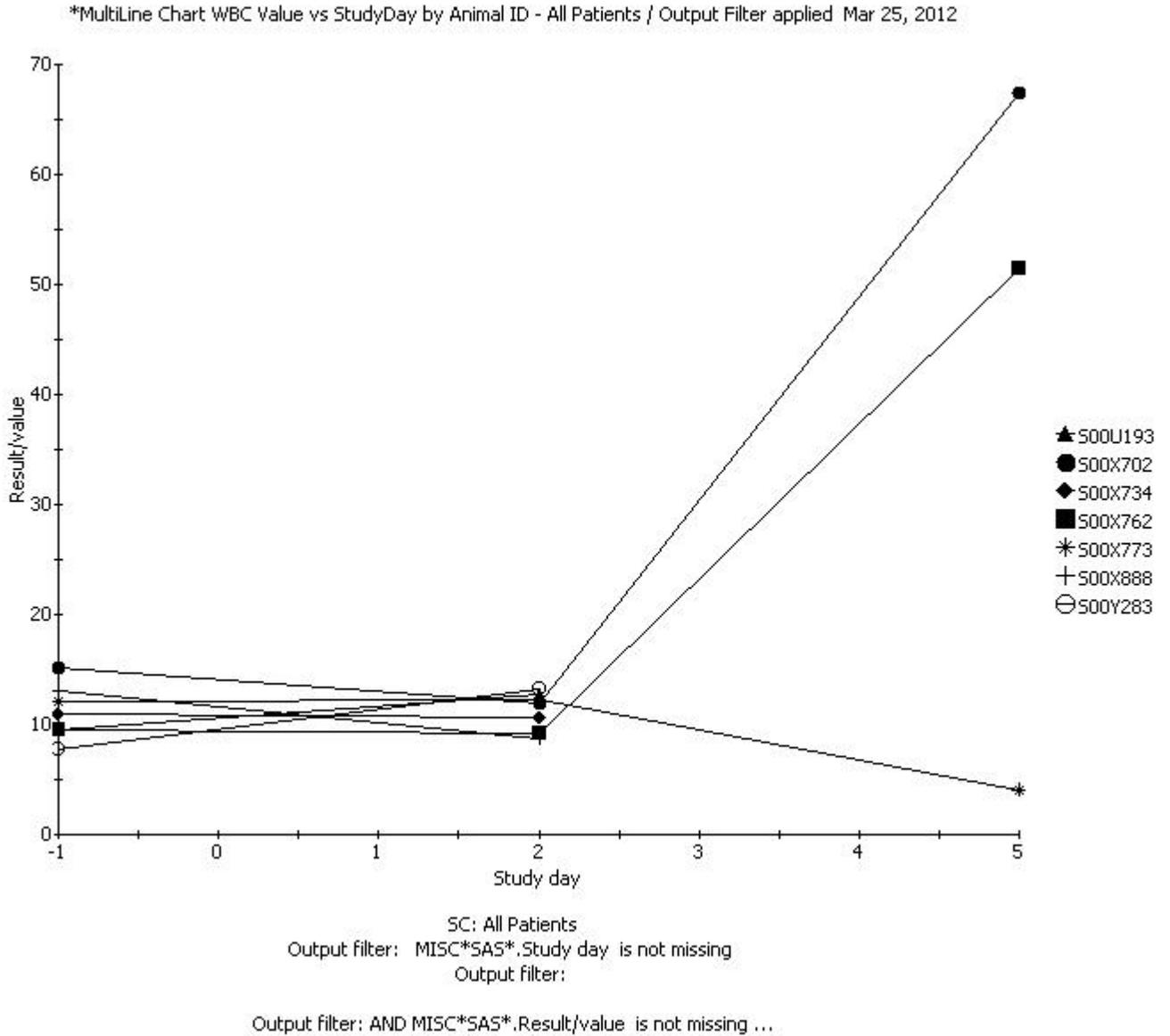
Laboratory Findings

Hematology Results

Blood samples for hematology and coagulation studies were collected at pre-study, Day 2, Day 6, and at the end of study, Day 28. A significant difference is found in the elevated total white blood cell (WBC) count in the control group at Day 5. Five animals had elevated WBC during the first six days post exposure to *Y. pestis*; two control animals (X702, X762) and three levofloxacin-treated animals (X663, X648, and Y226). The two control animals had elevated WBC with increased neutrophils on the day of death and two levofloxacin-treated animals had a return to baseline WBC at Day 28.

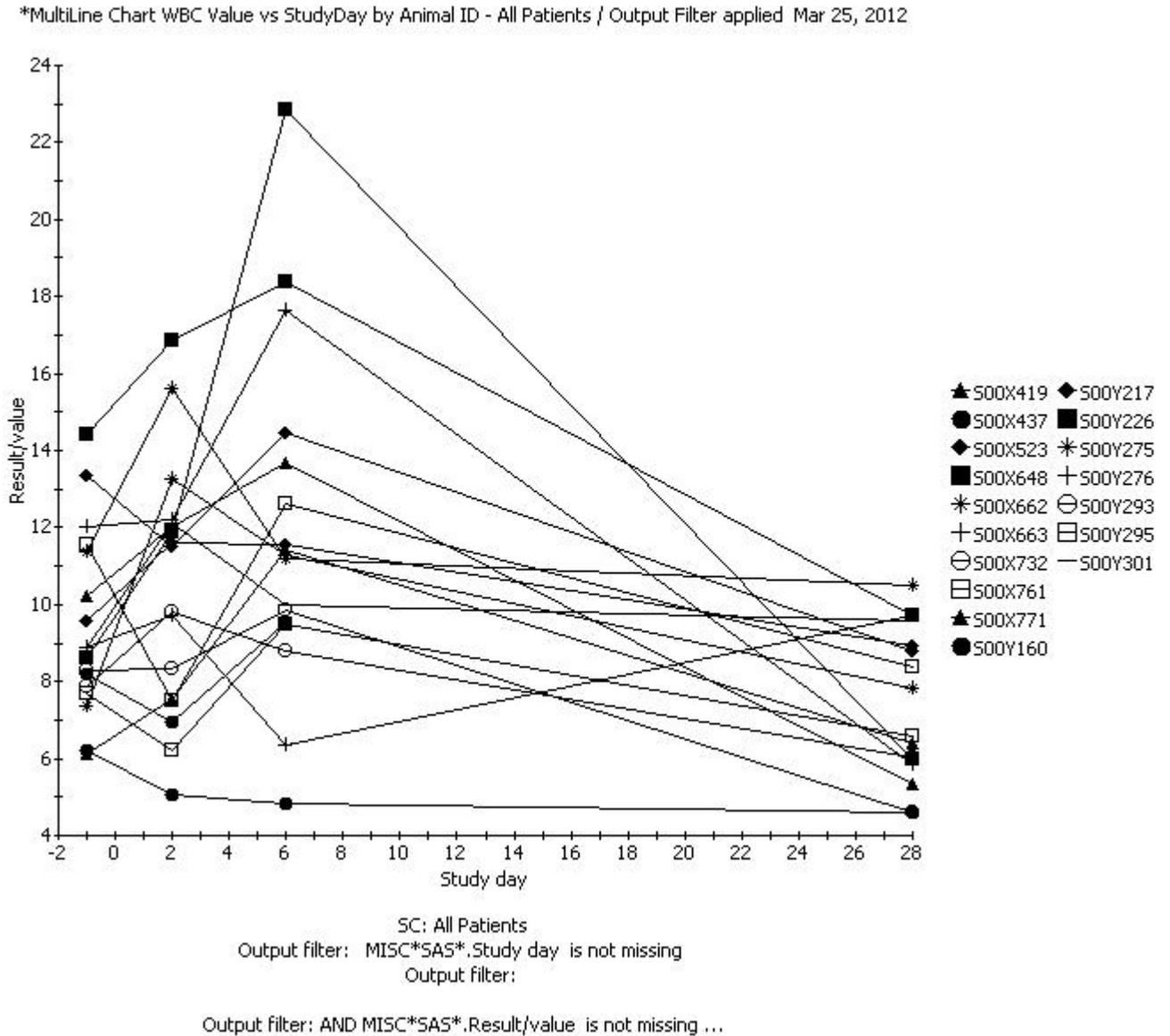
The WBC over the study period for placebo-treated animals and levofloxacin-treated animals are summarized in Figure 3 and Figure 4.

Figure 3. White Blood Cell Count over the Study Period for Placebo Animals



Analysis performed using JReview 9.2

Figure 4. White Blood Cell Count over the Study Period Levofloxacin-Treated Animals



Analysis performed using JReview 9.2

Among the red cell studies, significant increases in hematocrit in the control group were consistent with hemo-concentration due to dehydration in untreated disease. In the levofloxacin-treated group, the mean decreases in hematocrit by 7%, along with decreases in hemoglobin concentration, reticulocyte and red cell counts were consistent with a physiological impact of systemic infection on erythropoiesis. Increased D-dimer values were observed in two control animals and increased prothrombin times were observed in two animals. Levofloxacin-treated animals did not have evidence of disseminated intravascular coagulopathy based on fibrinogen levels and partial thromboplastin times.

Chemistry Results

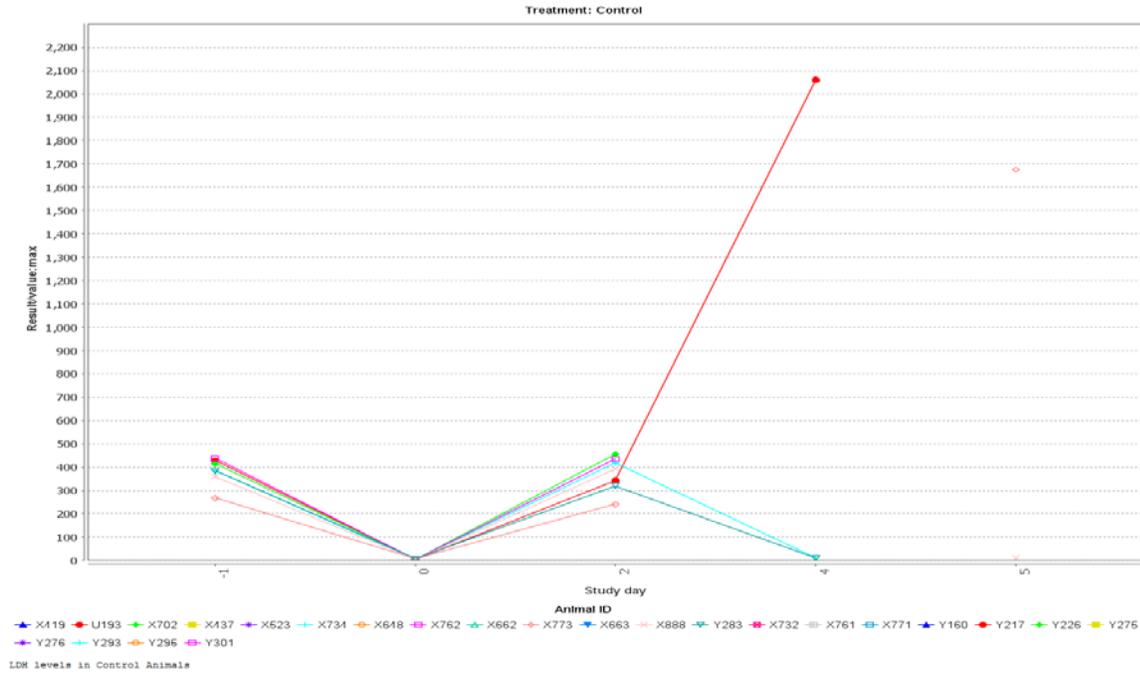
Blood samples for serum chemistries were collected at pre-study, Day 2, Day 6 and end of study, Day 28. Increases in serum enzymes alanine aminotransferase (ALT), alkaline phosphatase (APT), aspartate aminotransferase (AST), glutamyl transferase (GGT), lactate dehydrogenase (LDH) and total bilirubin, with decreases in total protein, cholesterol and triglycerides, were observed in control group animals prior to euthanasia (Day 4 to 5) compared to pre-study values. Increases in serum enzymes ALT, AST, LDH, total bilirubin, total protein, and triglycerides were observed in levofloxacin-treated animals on Day 6 compared to pre-study values.

Changes in electrolytes were not clinically significant. Blood glucose was decreased in both groups, and blood urea nitrogen (BUN) was increased in both groups, and creatinine increased in the control group. Only the elevations in BUN increase of 28 mg/dl and the glucose decrease of 50 mg/dL in the control group were clinically significant, consistent with more severe disease by Day 6.

Mean elevation of AST and LDH enzymes are approximately twice as high in the control compared to antibiotic-treated groups but this difference was not significant. Elevations in LDH and AST are consistent with lung inflammation. LDH values in levofloxacin-treated animal values returned to pre-study values by the end of the study, Figure 5 and Figure 6.

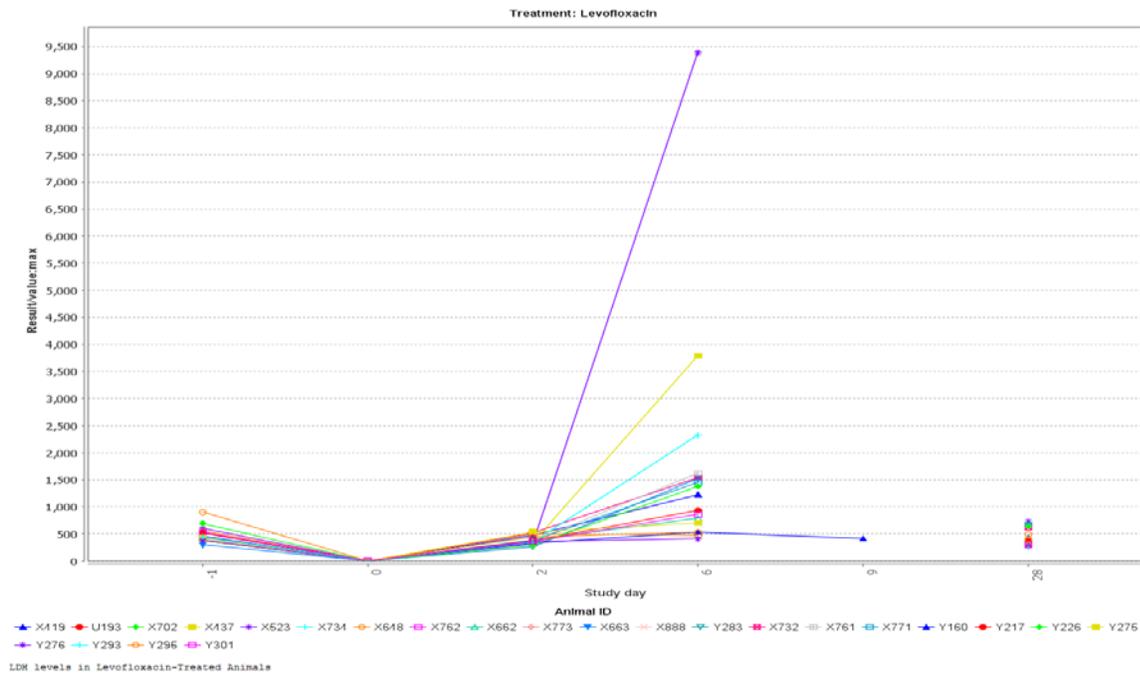
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Figure 5. LDH Levels in Control Animals



Analysis performed using JReview 9.1.4

Figure 6. LDH Levels in levofloxacin-Treated Animals



Analysis performed using JReview 9.2

Blood Gases

Arterial blood gas (ABG) tests were measured pre-study in Cohorts 1 and 2. Since ABG data were only obtained from Cohort 1 and 2 animals (prior to challenge) no comparisons were made. Pre-study samples from asymptomatic animals had low arterial oxygen partial pressures and hemoglobin saturation which may have been due to the difficulty in obtaining arterial blood from unanesthetized, restrained animals. ABG tests were scheduled for moribund animals in Cohorts 1 and 2 but were not technically possible probably due to hypotension.

Blood Cultures

Quantitative bacteriology was performed on blood samples pre-study and beginning Day 2 to Day 6 and then following challenge and periodically until the terminal blood culture on Day 28, Table 9.

Control Animals: All animals in the control group (X702, X773, X762, U193, X888, X734, Y283) were bacteremic with *Y. pestis*. Calculated bacterial colony counts in control animals ranged from 1.1×10^4 to greater than 3.0×10^5 CFU/mL at the end of life.

Levofloxacin-Treated Animals: Among the levofloxacin-treated animals, 12 of 17 (71%) had *Y. pestis* bacteremia detected prior to infusion. This number includes animal Y160 which was euthanized at Day 9 for a stomach complication. *Y. pestis* was detected in blood cultures in levofloxacin-treated animals after the start of infusions but was undetectable in all levofloxacin-treated animals by Day 6, Table 9. Four animals (X419, X732, X761, Y293-first 3 animals in cohort 2 and Y293 in cohort 3) were not bacteremic with *Y. pestis* during the study. One levofloxacin-treated animal (#Y293) had 1 colony of *Y. pestis* isolated from a pre-infusion blood sample on a qualitative culture plate on Day 3, all other catheter blood plates at this time point were contaminated with colonies inconsistent with *Y. pestis*. If Animal Y293 was included in the survival analysis for animals with *Y. pestis* bacteremia, the survival rate would be 92% (12/13), 95% CI (28.3%, 99.8%), P value 0.0007

Blood Cultures for all cohorts throughout the course of the study are summarized in Table 23.

Table 23. Blood Culture Results

Group	Treatment	Animal ID	Pre Study	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 14	Day 21	Day 28
1	Control	X702	BLD	BLD ^{>3 0x10⁵}	BLD	9.0x10 ³	>3.0x10 ⁵						
1	Control	X773	BLD ^{>3 0e+0³}	BLD ^{>2 0x10⁵}	BLD	1.1x10 ²	1.1x10 ⁴						
1	Control	X762	BLD	BLD	7.2x10 ⁴	2.8x10 ⁴	>3.0x10 ⁵						
1	Levofloxacin	X663	BLD ^{>3 0x10³}	BLD ^{>3 0x10⁵}	BLD	BLD	BLD	BLD	BLD		BLD	BLD	3.3
1	Levofloxacin	X662	BLD	BLD	56	BLD	BLD	BLD	BLD		BLD	BLD	BLD
1	Levofloxacin	X648	BLD	BLD	86	BLD	BLD	BLD	BLD		BLD ^{3 3}	BLD	6.6
1	Levofloxacin	X437	BLD	BLD	BLD	BLD	2.3x10 ³	BLD	BLD	BLD	BLD ^{1 2x10²}	BLD	BLD
1	Levofloxacin	X523	BLD	6.2x10 ⁴	BLD	BLD	BLD	BLD	BLD		BLD	BLD ^{>3 0x10³}	BLD ^{>3 0x10³}
2	Control	U193	BLD	BLD	3.0x10 ²	3.0x10 ⁵							
2	Control	X734	BLD	13	3.9x10 ⁴								
2	Levofloxacin	X732	BLD	BLD	BLD	BLD	BLD	BLD			BLD	BLD	BLD
2	Levofloxacin	X419	BLD	BLD	BLD	BLD	BLD	BLD			BLD	BLD	BLD
2	Levofloxacin	X771	BLD	BLD	8.0x10 ²	BLD	BLD	BLD			BLD	BLD	BLD
2	Levofloxacin	X761	BLD	BLD	BLD	BLD	BLD	BLD			BLD	BLD	BLD
3	Control	X888	BLD	BLD	BLD	1.5x10 ⁴							NS
3	Control	Y283	BLD	BLD	3.9x10 ²	BLD ^{3 7x10⁴}	>3.0x10 ⁵						
3	Levofloxacin	Y160	BLD	BLD	5.0x10 ³	BLD	BLD	BLD	BLD	BLD			
3	Levofloxacin	Y217	BLD	BLD	1.8x10 ²	BLD	BLD	BLD	BLD		BLD	BLD	BLD
3	Levofloxacin	Y226	BLD	BLD	1.8x10 ²	BLD	BLD	BLD	BLD		BLD	BLD	BLD
3	Levofloxacin	Y295	BLD	BLD	BLD	BLD	33	BLD	BLD		BLD	BLD	CATH*
3	Levofloxacin	Y275	BLD	BLD	BLD	9.2x10 ²	BLD	BLD	BLD	BLD	BLD	BLD	CATH*
3	Levofloxacin	Y276	BLD	BLD	BLD	BLD	BLD	BLD	4.9x10 ⁴	BLD	BLD	BLD	CATH*
3	Levofloxacin	Y293	BLD	BLD	BLD	BLD	BLD	BLD	BLD		BLD	BLD	CATH*

3	Levofloxacin	Y301	BLD	BLD	3.3x10 ²	BLD	BLD	BLD	BLD	BLD	BLD	CATH*
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BLD = below level of detection- negative blood culture

Green highlight = bacterial contamination (not *Y. pestis*) with superscript giving the colony count (if performed)

Gold highlight = animals with challenge dose < 20LD₅₀

Pink highlight = levofloxacin-treated animal, not bacteremic with *Y. pestis* at time of treatment

CATH= IV Catheter tip culture (culture positive with bacterial contaminants, not *Y.pestis*)

Tissue Cultures

Cultures were performed on selected tissues obtained at necropsy. Lung tissue samples were positive for *Y. pestis* in seven control animals. The tissue burden of *Y. pestis* was high (10^7 – 10^9 CFU/g) for control animals. Lung tissue samples obtained at necropsy from two animals in the levofloxacin-treated group were positive for *Y. pestis*. Animal X648 had a positive lung tissue (lesion) and a terminal blood culture positive for *Y. pestis*. X523 had a lung tissue culture positive for *Y. pestis* but a negative terminal blood culture.

Tissue culture results are summarized in Table 24.

Table 24. Blood (CFU/mL) and Tissue Culture (CFU/ g) Results for 24 AGMs Challenged with *Y. pestis* CO92 Strain at Death or Euthanasia

Cohort	Treatment	Animal ID	Blood	Spleen	Liver	TBLN	Lung - NL	Lung -L
1	Control	X702	$>3.0 \times 10^5$	3.24×10^7	3.93×10^8	8.78×10^9	3.03×10^9	2.43×10^9
1	Control	X773	1.14×10^4	4.47×10^6	8.32×10^8	3.52×10^9	4.89×10^8	6.53×10^9
1	Control	X762	$>3.0 \times 10^5$	3.00×10^7	1.86×10^8	3.09×10^9	3.79×10^8	9.61×10^9
1	Levofloxacin	X663	3.33×10^0				BLD	BLD
1	Levofloxacin	X662	BLD				BLD	BLD
1	Levofloxacin	X648	6.67×10^0				BLD ^{5.3x10²}	1.77×10^2
1	Levofloxacin	X437	BLD				BLD	BLD
1	Levofloxacin	X523	BLD ^{>3.0 x 10³}				3.02×10^2	BLD
2	Control	U193	2.96×10^5	6.86×10^7	1.11×10^8	1.68×10^9	2.81×10^7	1.53×10^{10}
2	Control	X734	3.90×10^4	2.04×10^9	6.28×10^8	2.07×10^9	4.29×10^8	1.05×10^{10}
2	Levofloxacin	X732	BLD				BLD	BLD
2	Levofloxacin	X419	BLD				BLD	BLD
2	Levofloxacin	X771	BLD				BLD	BLD ^{28.8 x 10⁴}
2	Levofloxacin	X761	BLD				BLD	BLD
3	Control	X888	1.51×10^4	8.82×10^9	2.32×10^8	7.47×10^9	1.46×10^9	1.44×10^{10}
3	Control	Y283	$>3.0 \times 10^5$	2.50×10^9	1.74×10^8	4.85×10^9	5.69×10^9	4.39×10^{10}
3	Levofloxacin	Y160	BLD	BLD	BLD	BLD	BLD	BLD
3	Levofloxacin	Y217	BLD				BLD	BLD
3	Levofloxacin	Y226	BLD				BLD	BLD
3	Levofloxacin	Y295	BLD				BLD	BLD
3	Levofloxacin	Y275	BLD				BLD	BLD
3	Levofloxacin	Y276	BLD				BLD	BLD
3	Levofloxacin	Y293	BLD	BLD	BLD	BLD	BLD	BLD
3	Levofloxacin	Y301	BLD				BLD	BLD

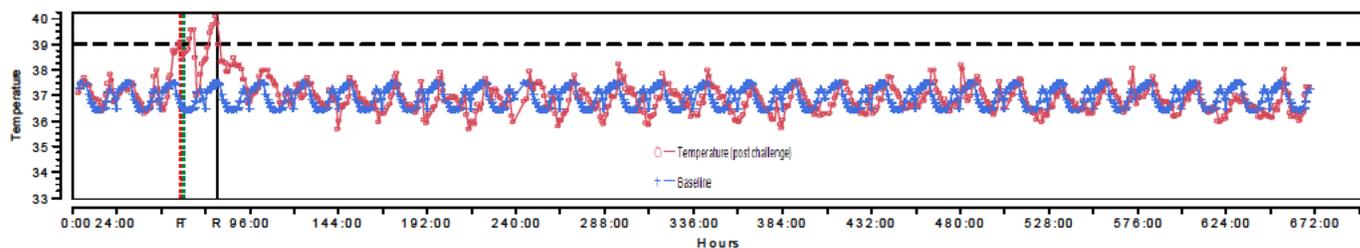
AGM= African Green Monkey; BLD = Below level of detection i.e. Negative Culture; CFU= Colony Forming Unit
 TBLN = Tracheobronchial lymph node; Lung-NL = Lung Non-Lesion; Lung-L = Lung Lesion
 Green highlight = Bacterial contaminant (not *Y. pestis*) with colony count, if performed (superscript);
 Pink highlight = Levofloxacin-treated animals that were not bacteremic with *Y. pestis* at the time of treatment;
 Gold highlight = Animals with challenge dose < 20 LD₅₀

Microbiologic Clearance of *Y. pestis* Infection

At Day 28, three animals had terminal blood cultures and/ or tissue cultures that were positive for *Y. pestis*. Two animals (X648, X663) had evidence of residual infection with *Y. pestis* in blood cultures. The three animals survived to Day 28.

Animal X663 was not positive for *Y. pestis* at the time of treatment or during treatment but had a terminal blood culture positive for *Y. pestis* (3 CFU/mL). All tissue cultures were negative for *Y. pestis*. One pre-study blood culture and Day 2 blood culture was contaminated with >300 CFU (contaminant not identified) as well as a lung tissue sample with an unknown contaminant at the end of study. A blood culture on Day 14 was negative for *Y. pestis*. The animal did not have a fever for several days prior to Day 28 as seen in the temperature tracing below. Bilateral interstitial infiltrate were observed on chest x-ray, described as mild, at Day 7 and it had a follow-up chest x-ray reported as normal at Day 28. Pulmonary histopathology showed resolving pneumonia.

Animal X663 - Temperature Pattern over Study Period

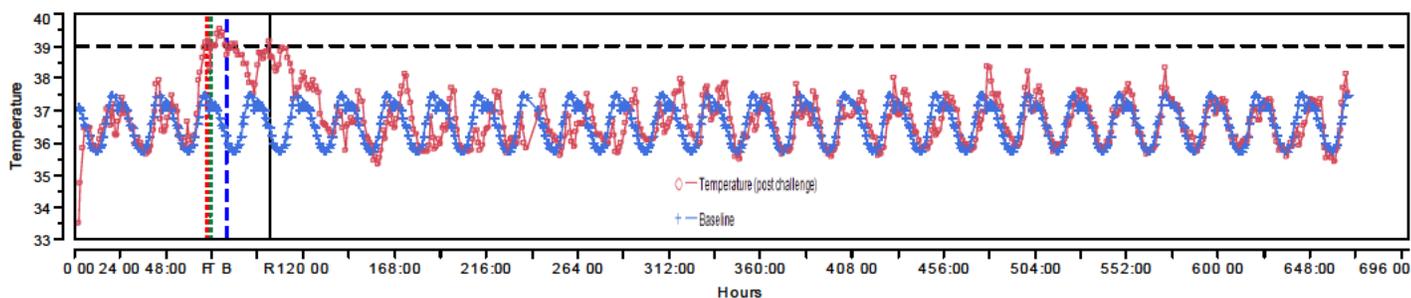


R= First infusion Levofloxacin

Clinical Reviewer's Comment: *Though Animal X663 appeared clinically well, it had bacteremia with a low colony count of *Y. pestis* at Day 28. *Y. pestis* in a blood culture is consistent with septicemic plague. Unfortunately, it was not possible to ascertain if the animal would become symptomatic if it had been followed beyond the end of the study.*

Animal X648 had a blood culture positive for *Y. pestis* at the time of treatment (87 CFU/mL), a terminal blood culture positive for *Y. pestis* (7 CFU/mL) on Day 28, and a positive lung (lesion) tissue culture for *Y. pestis* (177 CFU/g). On Day 14 post-challenge, a blood sample was contaminated (3 CFU/mL) as well as lung tissue sample at the end of study (530 CFU, contaminant not identified). The animal did not have a sustained fever for several days prior to Day 28 as seen in the temperature tracing below. A chest x-ray performed at Day 6 had bilateral interstitial infiltrates, described as moderate. A follow-up chest x-ray was not performed at Day 28. Pulmonary histopathology showed resolving pneumonia.

Animal X648 - Temperature Pattern over Study Period

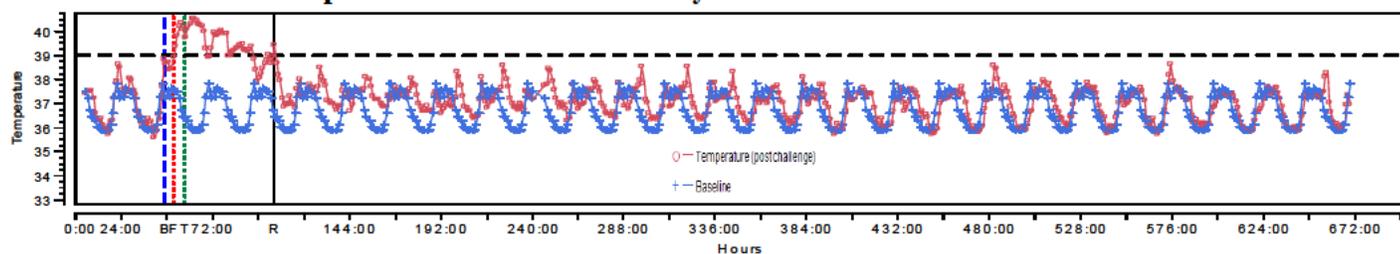


R= First infusion Levofloxacin

Clinical Reviewer's Comment: Though animal X648 appeared clinically well, it had bacteremia with *Y. pestis* at Day 28. *Y. pestis* in a blood culture is consistent with plague and unfortunately, it is not possible to ascertain if the animal would have been more symptomatic if it had been followed beyond the end of the study.

Animal X523 had a blood culture positive for *Y. pestis* at the time of treatment (Day 2 = 62,300 CFU/mL), a terminal blood culture negative for *Y. pestis*, and a positive lung (non-lesion) tissue culture positive for *Y. pestis* (302 CFU/g) at necropsy. Blood cultures on Day 3 through 7, and Day 14 were negative. On Day 21 and 28, a post-challenge blood sample was contaminated (>300 CFU/mL, contaminant not identified). The animal did not have a sustained fever for several days prior to Day 28 as seen in the temperature tracing below. A chest x-ray performed at Day 6 had bilateral interstitial infiltrate, described as moderate, and the animal had a normal chest x-ray with resolution of infiltrates at Day 28. The animal was euthanized and pulmonary histopathology showed resolving pneumonia.

Animal X523 - Temperature Pattern over Study Period



R= First infusion Levofloxacin

Clinical Reviewer's Comment: Animal X523 appeared to have recovered from pneumonic plague based on absence of sustained fever, a negative blood culture, and resolution of pulmonary infiltrates on the follow-up chest x-ray at Day 28. The animal had viable *Y. pestis* in a lung tissue culture however the animal's chest x-ray findings and pulmonary histopathology showed resolving pneumonia and the animal appeared clinically well.

Overall, the animals with positive blood cultures and or tissue cultures appeared well and appeared to have clinically recovered from pneumonic plague. They appeared to be handling the

infection and may have been experiencing transient bacteremia, however, it was not possible to ascertain if the animals would have become more symptomatic if they had been followed beyond the end of the study.

*The finding of *Y. pestis* in blood cultures from two animals X663 and X648 at the end of the study is concerning and suggests that a 10 day treatment course of IV levofloxacin may not be long enough to completely achieve microbiological clearance of *Y. pestis*.*

Radiologic Findings

An independent review of the chest radiographs was performed by a veterinarian, board certified in veterinary radiology/ radiation oncology). The radiologist was blinded to the study results. A summary of the chest x-ray reports were submitted for review.

Baseline chest X-rays were all reported as normal for all animals in the study. Eight animals in cohort 1 (n=8) and cohort 2 (n=4) had follow up chest X-rays taken post-aerosol challenge with *Y. pestis*. Animals in Cohort 3 had radiographs obtained only prior to aerosol challenge, except for one animal (Y160) that developed gastric necrosis of uncertain etiology and was euthanized and necropsied nine days after challenge.

Animals in Cohorts 1 and 2 had follow-up chest radiographs to evaluate for resolution of pulmonary infiltrates. Bilateral or unilateral pulmonary interstitial infiltrate(s) indicative of pneumonia was a consistent finding in all animals. The three control animals developed severe bilateral pulmonary interstitial infiltrates. In the nine animals which were treated with levofloxacin and survived in Cohorts 1 and 2, all chest radiographs taken on Days 5 or 6 of infection revealed abnormalities consistent with mild or moderate pulmonary infiltrates. Six of the nine animals had repeat chest x-rays at Day 28 that were reported as normal.

Among the nine levofloxacin-treated survivors, four animals (X419, X732, X761, X771) with pulmonary infiltrates had negative blood cultures. Five animals with radiological evidence of pneumonia developed bacteremia. These animals with evidence of pneumonic and septicemic plague survived with resolution of infiltrates on their chest X-rays and negative blood cultures therefore providing further support for the efficacy of levofloxacin for the treatment of plague. Pulmonary radiology results for control and levofloxacin-treated animals are summarized in Table 25.

Table 25. Pulmonary Radiology - Control and Levofloxacin-Treated Animals

			<i>Y. pestis</i>	Blood culture	Chest X-Ray	Chest X-ray Findings
Cohort	Animal ID	Treatment	LD ₅₀ equivalents	<i>Y. pestis</i> +/-	Baseline	Day of Study
1	X437	Levofloxacin	40	+	Normal	Diffuse bilateral interstitial infiltrate – mild @ Day 6
						No x-ray on Day 28.
1	X523	Levofloxacin	75	+	Normal	Bilateral interstitial infiltrate –moderate @

			<i>Y. pestis</i>	Blood culture	Chest X-Ray	Chest X-ray Findings
Cohort	Animal ID	Treatment	LD ₅₀ equivalents	<i>Y. pestis</i> +/-	Baseline	Day of Study
						Day 6
						Normal 4/22/08 (Day 28)
1	X648	Levofloxacin	57	+	Normal	Bilateral interstitial infiltrate –moderate @ Day 6
						No x-ray on Day 28.
1	X662	Levofloxacin	81	+	Normal	Bilateral interstitial infiltrate – mild @ Day 6
						No x-ray on Day 28.
1	X663	Levofloxacin	66	-	Normal	1. Bilateral interstitial infiltrate mild@ Day 7. 3/31/08
						2.Normal 4/22/08 (Day 28)
1	X702	Control	56	+	Normal	Bilateral interstitial infiltrate - severe (@ day 5. Day 5: Died
1	X762	Control	76	+	Normal	Bilateral interstitial infiltrate (R>L) – severe @ day 5. Day 5:Euthanized
1	X773	Control	143	+	Normal	Bilateral interstitial infiltrate (L>R) – severe @ day 5. Day 5: Euthanized
2	U193	Control	121	+	Normal	ND; Day 4:Euthanized
2	X419	Levofloxacin	120	-	Normal	Interstitial infiltrate primarily R lung, ventral – moderate @ Day 5
						Normal 5/30/08 (day28)
2	X732	Levofloxacin	124	-	Normal	Bilateral interstitial infiltrate –moderate @ Day 6
						Normal 5/30/08 (Day 28)
2	X734	Control	145	+	Normal	ND; Day 4: Died
2	X761	Levofloxacin	118	-	Normal	Interstitial infiltrate primarily R lung, ventral – mild @ Day 5
						Normal 5/30/08 (Day 28)
2	X771	Levofloxacin	117	+	Normal	Bilateral interstitial infiltrate (R>L) – moderate, @ Day 5
						Normal 5/30/08 (Day 28)
3	X888	Control	44	+	Normal	ND
3	Y160	Levofloxacin	6	+	Normal	Bilateral interstitial infiltrate (R>L) – moderate, @ day 9; Day 9: Euthanized
3	Y217	Levofloxacin	38	+	Normal	ND
3	Y226	Levofloxacin	12	+	Normal	ND
3	Y275	Levofloxacin	4	+		
3	Y276	Levofloxacin	3	+	Normal	ND
3	Y283	Control	47	+	Normal	ND
3	Y293	Levofloxacin	62	-	Normal	ND
3	Y295	Levofloxacin	3	+	Normal	ND
3	Y301	Levofloxacin	3	+	Normal	ND

ND= Not done

Analysis performed using JReview 9.2

Clinical Reviewer's Comment: *Animals were on treatment when the chest x-rays were taken at study Day 5 or 6. The finding of pulmonary infiltrates on chest x-ray indicates that plague pneumonia had progressed to become radiographically apparent despite antibacterial treatment initiated prior to the x-ray. Additionally, one can conclude that treatment with levofloxacin was efficacious in spite of being initiated after the onset of radiographically apparent pneumonia. The resolution of the pulmonary infiltrates in nine levofloxacin-treated animals support the efficacy of levofloxacin IV for the treatment of pneumonic plague. Five of the nine animals with radiological evidence of plague pneumonia developed bacteremia and these animals with more severe disease (pneumonic and septicemic plague) survived and had resolution of their chest x-rays and clearance of their blood cultures. These results provide additional support for the efficacy of levofloxacin for the treatment of plague.*

Necropsy Findings

Samples of formalin fixed collected tissues were designated for histopathologic examination. Tissues were processed routinely, paraffin embedded, sectioned and stained with hematoxylin and eosin. Tissues samples were taken fresh for microbial quantitation or fixed in 10% neutral buffered formalin (brain tissue was fixed for histopathologic exam only). Histopathologic lesions were graded subjectively by a single pathologist on a scale of 1 to 4 (1=minimal, 2=mild, 3=moderate, 4=marked).

Twenty-four animals (7 controls and 17 levofloxacin-treated) underwent necropsy and histopathological examination of tissues. Lungs, lymph nodes, liver, spleen, heart, brain, and stomach were examined.

A summary of the histopathologic findings was prepared for the applicant by a veterinary pathologist [REDACTED] (b)(4) and are summarized below.

Gross Pathology

Control Animals: The main lesions were 1) large deep purple regions in multiple lobes of the lungs, which were described as being consistent with extensive parenchymal hemorrhage and 2) enlarged and discolored tracheo-bronchial lymph nodes. Other lesions included discolored liver and spleen without enlargement, an enlarged heart in two animals and fluid on the brain (histopathology: mildly inflamed meninges) of one animal.

Levofloxacin-Treated Animals: In survivors, the lungs had a discolored surface consistent with anesthesia artifact or resolution of plague pneumonia.

Histopathologic Findings

The relevant pulmonary microscopic histopathologic lesions are summarized in the following table and are discussed below.

**Selected Microscopic Lesion Incidence Summary Table
 (All Cohorts)**

<i>Path/Tox group designation</i>	Males		Females	
	<i>Controls (1,3,5)</i>	<i>Levo (2,4,6)</i>	<i>Controls (1,3,5)</i>	<i>Levo (2,4,6)</i>
<i>Number examined</i>	4	8	3	9
<i>Found moribund or dead</i>	4	0	3	1 ^a
Lungs				
Inflammation, fibrinosuppurative and hemorrhagic with bacteria	4 [3.8]	0 [0.0]	3 [3.7]	0 [0.0]
Inflammation, histiolympocytic (septal infiltrates)	0 [0.0]	7 [1.5]	0 [0.0]	6 [1.2]

Number in brackets represents average severity score (sum of severity scores/number of animals in group).

^aEuthanized moribund with vomiting.

Source: Study Report, FY07-070

Control Animals: Tissue inflammation, fibrino-suppurative, and hemorrhage with bacteria (consistent with *Y. pestis*) indicative of fibrino-suppurative pneumonia were observed in the seven control animals. Overall, the findings were “moderate” to “marked” in severity. Pleural fibrosis (minimal severity) was associated with telemeter placement in three animals. Other lesions noted included edematous tracheo-bronchial lymph nodes with bacteria typical of *Y. pestis* and hemorrhage (mild severity). Periportal inflammation (minimal severity) in the liver was observed in all controls. Splenic congestion with bacteria (minimal severity) was observed in a few control animals and in no levofloxacin-treated animals.

Levofloxacin-Treated Animals: The histopathological findings in the lungs of levofloxacin-treated animals were more chronic in nature. Chronic lesions of septal histio-lymphocytic infiltrates within the pulmonary parenchyma were seen in 13 of the levofloxacin-treated animals and were interpreted as ongoing resolution of the effects of an initial pulmonary infection. These findings were supported by resolution of high fever and decrease in respiratory rates after 2 to 3 days on levofloxacin treatment seen in the survivors. Additionally, resolution of pulmonary infiltrates were observed on chest x-rays of those levofloxacin-treated animals that had follow-up chest x-rays (n=6) at end of study at Day 28. Sinusoidal leukocytosis was observed in tracheo-bronchial lymph nodes (minimal severity) in one animal. Sinusoidal inflammation (minimal severity) as random foci in the liver was observed in eight levofloxacin-treated animals. Amyloid (minimal severity) was observed in splenic tissue from one levofloxacin-treated animal. One male animal in the levofloxacin-treated group had evidence of inflammation of the meninges (minimal severity).

Clinical Reviewer's Comment:

The histopathological findings in the pulmonary parenchyma were consistent with bacterial bronchopneumonia. The histopathological findings of fibrino-suppurative pneumonia in control animals were similar to the pulmonary histopathological findings described for pneumonic plague in the natural history studies in AGM. The bacterial rods seen on H&E stains were consistent with Y. pestis.

The histopathological findings in levofloxacin-treated animals were consistent with resolving pneumonia. Chronic septal pulmonary inflammation and mixed liver inflammation changes were interpreted by the pathologist as resolving lesions after infection with Y. pestis and subsequent treatment with levofloxacin.

The clinical findings in survivors were consistent with resolving pneumonia, i.e. resolution of high fever and decrease in respiratory rates after 2 to 3 days on levofloxacin treatment. Additionally, resolution of pulmonary infiltrates were observed in the levofloxacin-treated animals that had follow-up chest x-rays (n=6) at the end of study, i.e. Day 28.

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/s/

ELIZABETH M OSHAUGHNESSY
04/09/2012

JOHN J ALEXANDER
04/10/2012

CLINICAL FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	NA	Comment
21.	For chronically administered drugs, have an adequate number of patients (based on ICH guidelines for exposure ¹) been exposed at the dose (or dose range) believed to be efficacious?			X	
22.	For drugs not chronically administered (intermittent or short course), have the requisite number of patients been exposed as requested by the Division?			X	
23.	Has the applicant submitted the coding dictionary ² used for mapping investigator verbatim terms to preferred terms?			X	
24.	Has the applicant adequately evaluated the safety issues that are known to occur with the drugs in the class to which the new drug belongs?			X	
25.	Have narrative summaries been submitted for all deaths and adverse dropouts (and serious adverse events if requested by the Division)?			X	
OTHER STUDIES					
26.	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X			
27.	For Rx-to-OTC switch and direct-to-OTC applications, are the necessary consumer behavioral studies included (<i>e.g.</i> , label comprehension, self selection and/or actual use)?			X	
PEDIATRIC USE					
28.	Has the applicant submitted the pediatric assessment, or provided documentation for a waiver and/or deferral?	X			Submitted Dec 23, 2011
ABUSE LIABILITY					
29.	If relevant, has the applicant submitted information to assess the abuse liability of the product?			X	
FOREIGN STUDIES					
30.	Has the applicant submitted a rationale for assuming the applicability of foreign data in the submission to the U.S. population?			X	
DATASETS					
31.	Has the applicant submitted datasets in a format to allow reasonable review of the patient data?	X			
32.	Has the applicant submitted datasets in the format agreed to previously by the Division?		X		
33.	Are all datasets for pivotal efficacy studies available and complete for all indications requested?		X		
34.	Are all datasets to support the critical safety analyses available and complete?		X		
35.	For the major derived or composite endpoints, are all of the raw data needed to derive these endpoints included?		X		#32-34. The natural history and pivotal

¹ For chronically administered drugs, the ICH guidelines recommend 1500 patients overall, 300-600 patients for six months, and 100 patients for one year. These exposures MUST occur at the dose or dose range believed to be efficacious.

² The “coding dictionary” consists of a list of all investigator verbatim terms and the preferred terms to which they were mapped. It is most helpful if this comes in as a SAS transport file so that it can be sorted as needed; however, if it is submitted as a PDF document, it should be submitted in both directions (verbatim -> preferred and preferred -> verbatim).

File name: 5_Clinical Filing Checklist for NDA_BLA or Supplement 010908

CLINICAL FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	NA	Comment
					efficacy (FY07-070) studies were missing information in the datasets. Information requests were sent to the sponsor, see correspondence dated 12/7/2011 and 12/27/2011
CASE REPORT FORMS					
36.	Has the applicant submitted all required Case Report Forms in a legible format (deaths, serious adverse events, and adverse dropouts)?			X	
37.	Has the applicant submitted all additional Case Report Forms (beyond deaths, serious adverse events, and adverse drop-outs) as previously requested by the Division?			X	
FINANCIAL DISCLOSURE					
38.	Has the applicant submitted the required Financial Disclosure information?		X		No financial disclosure submitted because no clinical studies are required to support the NDA
GOOD CLINICAL PRACTICE					
39.	Is there a statement of Good Clinical Practice; that all clinical studies were conducted under the supervision of an IRB and with adequate informed consent procedures?			X	One natural history study (A03-09G) was not conducted under GLP procedures.

IS THE CLINICAL SECTION OF THE APPLICATION FILEABLE? _____ Yes_

If the Application is not fileable from the clinical perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

1. Infusion dataset: The parameter "Study Day" was provided in the other study datasets. Provide the "Study Day" in the infusion dataset. Provide the "Date and Time" as separate variables.
2. Animal ID dataset: Verify if the study start date is the "end of nebulization date." Provide the "Date and Time" as separate variables and the "Study Day" for death and fever onset.

File name: 5_Clinical Filing Checklist for NDA_BLA or Supplement 010908

CLINICAL FILING CHECKLIST FOR NDA/BLA or Supplement

Reviewing Medical Officer

Date

Clinical Team Leader

Date

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ELIZABETH M OSHAUGHNESSY
01/04/2012

JANICE K POHLMAN
01/04/2012