

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

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

MICROBIOLOGY / VIROLOGY REVIEW(S)

Division of Anti-Infective Products

Clinical Microbiology Review

NDA#: 20-634, 20-635, 21-721
Levofloxacin
Janssen Pharmaceuticals

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Efficacy Supplement
Date Completed: 4/5/2012

Reviewer: Simone M. Shurland

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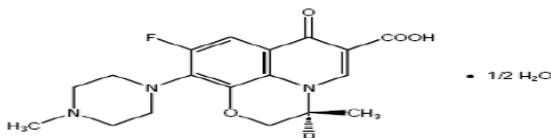
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SPONSOR

Johnson & Johnson Pharmaceutical Research & Development, L.L.C.
Janssen Pharmaceuticals, Inc.
920 Route 202 South
Raritan, NJ 08869-0602

DRUG PRODUCT NAME

Proprietary Name Levaquin®
Non-Proprietary Name Levofloxacin
Chemical Name (-)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemihydrate
Molecular Weight 370.38
Molecular Formula C₁₆H₁₄F₃N₅O
Structural Formula



DRUG CATEGORY

Anti-bacterial

PROPOSED INDICATION

Treatment of plague

PROPOSED DOSAGE FORM, STRENGTH, ROUTE OF ADMINISTRATION AND DURATION OF TREATMENT

Dosage Form	Oral	Intravenous
Dose Strength	500 mg ^{a,b}	500 mg

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Frequency	24 hours ^{a,b}	24 hours
Duration	14 days	14 days

^aFor pediatric patients >50 kg and > 6 months of age; dose 500 mg, every 24 hours, 14 days

^bFor pediatric patients <50 kg and > 6 months of age; dose 8 mg/kg, every 12 hours, 14 days

DISPENSED

Prescription Product

TYPE OF SUBMISSION:

Efficacy Supplement

PURPOSE OF SUBMISSION

New Indication-Treatment of pneumonic plague following exposure to *Yersinia pestis* in adults and pediatric patients

RELATED DOCUMENTS

IND#36,627, IND# 38,368, IND#64,429

RECOMMENDATIONS

The efficacy supplement is approvable with respect to microbiology pending an accepted version of the labeling.

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EXECUTIVE SUMMARY

Yersinia pestis is a nonsporulating, nonmotile, pleomorphic, Gram-negative coccobacillus that shows bipolar (also termed safety pin) staining with Wright, Giemsa, or Wayson stain. *Y. pestis* is a lactose nonfermenter, urease and indole negative, and belongs to the *Enterobacteriaceae* family. The organism grows on a wide variety of common microbiologic media (e.g. brain heart infusion, sheep blood agar and MacConkey agar); however, grows optimally at 28°C typically requiring 48 hours for observable growth. The bacteria elaborate a lipopolysaccharide endotoxin, coagulase, and a fibrinolysin in addition to a number of other virulence important for plague pathogenesis. The genes encoding these virulence factors reside primarily on 3 plasmids one of which is the F1 antigen which is encoded on the pFra plasmid. The F1 component is also responsible for eliciting a humoral immune response.

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. These type II topoisomerases play essential roles in bacterial DNA replication, transcription, repair, and recombination.

Antimicrobial Spectrum of Activity

Levofloxacin has been shown to have *in vitro* activity against *Y. pestis* laboratory clinical isolates with MIC values that range from <0.03 to 0.12 µg/mL. There were more than 189 isolates tested which were evaluated in 5 studies. There was no significant difference in the MICs of the *Y. pestis* tested by biovar type (i.e., Antiqua, Medievalis, Orientalis) or geographic region. There were no wild type *Y. pestis* isolates with a MIC greater than 0.12 µg/mL.

The *Y. pestis* CO92 strain, used in the pivotal efficacy study, had MICs of 0.03 µg/mL across laboratories; this MIC value was similar to the MICs observed of *Y. pestis* isolates sourced from various geographic countries.

Mechanism of Resistance

Resistance to levofloxacin typically results by either mutations in the genes bacterial topoisomerase II enzymes, decreased outer membrane permeability or drug efflux. No specific modifying or degrading enzymes have been found to be a mechanism of bacterial resistance to levofloxacin or other fluoroquinolones. Against the *Enterobacteriaceae* organisms resistance to levofloxacin develops slowly by multiple step mutations. The frequency of spontaneous mutations *in vitro* to levofloxacin generally occur at a frequency between 10^{-9} to 10^{-6}. The mutation frequency for *Y. pestis* ΔCO92 st [REDACTED] (b) (4) range of the mutation frequency reported in the levofloxacin labeling [REDACTED]

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There is no known cross-resistance between levofloxacin and other classes such as penicillins, cephalosporins, aminoglycosides, macrolides and tetracyclines in which *Y. pestis* resistance have been reported.

Activity *in vivo*

Several studies evaluated the activity of prophylactic and therapeutic levofloxacin in mice and rat models of experimental pneumonic plague. Overall, the results demonstrated that levofloxacin dosed once daily for 6 days at 5 to 15 mg/kg/day 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.

Pneumonic Plague

A randomized, placebo-controlled animal study in African Green monkeys challenged via aerosol to a mean dose of 65 LD₅₀ (range, 3 to 145 LD₅₀) of *Y. pestis* strain CO92 was evaluated under the Animal Rule (21 CFR 314.610). The MIC of levofloxacin for the *Y. pestis* strain used in the study is 0.03 µg/mL. A dosing regimen of levofloxacin was designed to approximate the human AUC value obtained with a 500 mg daily dose of levofloxacin. Animals were administered 8 mg/kg levofloxacin followed by a second infusion within 12 hours of 2 mg/kg levofloxacin. The treatment regimen was given over a maximum of 10 days for a total of 20 doses. All of the control animals died within 5 days post-challenge. Seventeen of the 18 levofloxacin treated animals survived. The levofloxacin treated monkey that did die, succumbed on day 9 post-challenge. C_{max} concentrations of levofloxacin after the 1st and 3rd doses of 8 mg/kg ranged from 2.4 to 4.5 µg/mL, which were above the 0.03 µg/mL MIC value of the *Y. pestis* strain used.

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1. INTRODUCTION AND BACKGROUND

Levofloxacin is a fluoroquinolone, an antibacterial agent that exhibits bacterial activity against most Gram positive and Gram negative pathogens. In this submission, the applicant is requesting a priority review of the supplemental New Drug Application (sNDA) levofloxacin (tablets, injection and oral solution) for the proposed indication of treatment of pneumonic plague following exposure to *Yersinia pestis* in adults and pediatric patients' ≥ 6 months of age.

1.1. Levofloxacin

Levofloxacin was approved in 1996 and marketed as Levaquin® by J&J Pharmaceuticals for the oral tablet (NDA 20-634), intravenous (NDA 20-635) and oral suspension (NDA 21-721) formulation. Levofloxacin is the L-isomer of the racemate, ofloxacin, a quinolone antimicrobial agent. The antimicrobial activity of ofloxacin resides primarily in the L-isomer. Levofloxacin has been shown to be active for the treatment of a wide range of infections including nosocomial pneumonia, community acquired pneumonia, acute bacterial sinusitis, acute bacterial exacerbation of chronic bronchitis, complicated skin and skin structure infections, uncomplicated skin and skin structure infections, chronic bacterial prostatitis, complicated urinary tract infections and acute pyelonephritis post-exposure inhalational anthrax. Levofloxacin is also approved for the treatment of inhalational anthrax (post-exposure), a disease with similar pathophysiology as plague to reduce the incidence or progression of disease following exposure to aerosolized *Bacillus anthracis* spores. Levofloxacin is available in both intravenous (IV) and oral formulations which allow the benefits of early switching from parenteral to oral therapy. The approved dosage and administration of levofloxacin for the tablets or oral solution formulation is 250 mg, 500 mg, or 750 mg administered orally every 24 hours, as indicated by the type of infection. For instance, the 500 mg dose administered once daily for 7 – 14 days is recommended for community acquired pneumonia or higher dosing of 750 mg once daily for 5 days for severer cases. The 750 mg once daily dose administered for 7 – 14 days is recommended for nosocomial pneumonia.

1.2. Plague

Plague is a zoonotic disease caused by the bacterium *Yersinia pestis*. In nature, both wild and domestic rodents can serve as the natural reservoir for plague. Transmission to humans occurs primarily following the bites of fleas from infected rodents¹⁻⁸. Infection in humans is incidental, resulting from intrusion of persons into the natural wild cycle through direct contact with infected animals or through a break in the skin. Non-rodent mammals including squirrels, marmots, and carnivores such as domestic cats, prairie dogs, and coyotes have been found to be infected either by the bite of an infected flea or by direct contact with infectious tissues.

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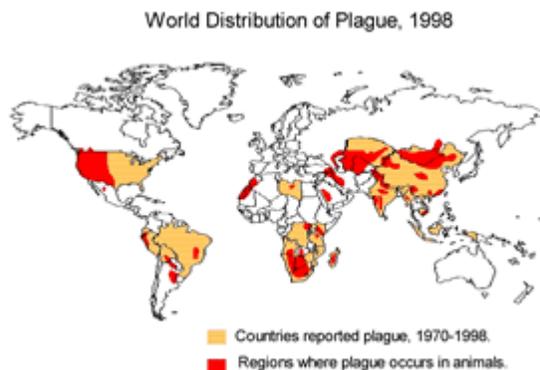
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1.2.1. Epidemiology

Globally, the World Health Organization (WHO) reports 1,000 to 3,000 cases of plague every year¹⁴. Endemic plague foci persist in areas such as East and South Africa, Mongolia and North China, northeastern part of South America and southwestern parts of the United States (Figure 1). In the most recent 15-year reporting period (1989 – 2003), over 80% of cases reported by the WHO were from countries in Africa. In the United States, the number of states reporting cases of human plague increased from 3 in the 1950s to 13 in the 1990s.¹⁵ From 1990 to 2005, a total of 107 cases of plague were reported in the United States usually occurring in rural areas of the Western States. Plague is considered a biological threat agent, classified as a Category A agent by the Centers for Disease Control (CDC) due to its high case fatality rate, potential for mass production, aerosol dissemination to a large population, and history of prior use as a biological weapon.¹⁻⁸

Figure 1: World Distribution of Plague, 1998



Source: <http://www.cdc.gov/ncidod/dvbid/plague/world98.htm>

1.2.2. Clinical Signs and Symptoms

Plague in humans is usually manifested in one of three forms: bubonic, septicemic and pneumonic. The majority of cases are bubonic. **Bubonic plague** has an incubation period of 1 – 7 days and is characterized by rapid onset of high fever and swollen, tender lymph glands called “buboes”. **Septicemic plague** occurs when *Y. pestis* invades and multiply in the bloodstream this can occur secondary to bubonic plague and develop without detectable lymphadenopathy. Untreated septicemic plague is fatal in over 80% - 100% of cases; untreated bubonic plague is fatal in over 50% of cases. The bubonic and septicemic forms of plague do not spread from person to person.

Pneumonic plague is the least common but most fulminant and highly contagious form of plague. It can develop as a secondary complication of septicemic plague or results from inhalation of aerosols containing *Y. pestis* organisms. A review by Franz *et al.* (1997)¹³ reported that by inhalation an estimated infectious dose can be as low as 100 to 500 organisms. Reports of pandemic cases of primary pneumonic plague have shown that the

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incubation period is usually 2 – 4 days, rarely longer. The onset is often sudden with chills, fever, headache, generalized body pains, weakness, chest discomfort and cough with blood-tinged sputum. Gastrointestinal symptoms such as nausea, vomiting, abdominal pain and diarrhea may also develop. The disease progresses rapidly and leads to either infectious pneumonitis or lobar pneumonia. If chest x-rays are performed, radiographic findings are variable but may indicate localized pulmonary infiltrate or consolidation in the early stage, followed by a rapidly developing consolidation of segments and/or lobes of the same or opposite lung. The fatality rate is very high unless treatment is initiated within 18 to 24 hours after onset of disease¹⁻⁸.

1.2.3. Diagnosis

Diagnosis and confirmation of plague requires laboratory testing. Specimens for smears and culture include blood, lymph node aspirate in those with suspected buboes, sputum samples or tracheal aspirates in patients with suspected pneumonic plague and cerebrospinal fluid in those with suspected meningitis. Recovery and identification of *Y. pestis* culture from a patient sample is optimum for confirmation. *Y. pestis* is a Gram-negative, facultative anaerobic, non-motile, non-sporulating, coccobacilli belonging to the family *Enterobacteriaceae*. On polychromatic staining using Wright, Giemsa or Wayson stains, plague bacilli in clinical specimens demonstrate a characteristic bipolar appearance, often resembling closed safety pins. The organism grows on a wide variety of common microbiologic media (e.g. brain heart infusion broth, sheep blood agar and MacConkey agar); however grows optimally at 28°C, typically requiring 48 hours for observable growth.

Y. pestis possess several virulence factors essential for its infectivity and survival in the mammalian host.^{4,7} Molecular analysis of the genome showed that the majority of *Y. pestis* strains have a chromosome that consists of 4.65 million bases, as well as 3 plasmids. The genes encoding these virulence factors reside primarily on the 3 plasmids which include:

- **9.6 kb pesticin plasmid** (pPst also called pPCP1) which encodes for a plasminogen activator (*Pla*), a bacteriocin (*pesticin*) and a murine toxin (*Ymt*). Plasminogen activator is a protease that can activate plasminogen, a blood protein involved in the degradation of fibrin (fibrinolysin). The pesticin is a bacteriocin thought to be important for iron uptake by *Y. pestis* in mammalian hosts. The murine toxin (*Ymt*) expresses phospholipase D which have been shown to have a role in the transmission of plague in mice and rats but has no activity in other mammalian hosts.
- **70 kb low calcium response plasmid** (Lcr; also known as pCD1 or pYV), encodes gene products that are responsible for a group of virulence factors under low calcium conditions and are expressed at 37°C. These factors include 11 surface outer membrane proteins (Yops) and a soluble V antigen. The Yops proteins (Yop H, Yop E and Yop M) interfere with immune cell function and can cause immune

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cell death by apoptosis. The V antigen prevents recruitment of inflammatory cells and granuloma formation which would usually end an infection.

- **110 kb pFra plasmid** contains a structural gene (*cafI*) which encodes an anti-phagocytic polypeptide, fraction 1 protein (F1). The F1 antigen is exported to the cell surface, where it forms a fibrillar capsule that inhibits phagocytosis by neutrophils and macrophages in synergy with the activity of the type III secretion system. The F1 component is also responsible for eliciting a humoral immune response. Detection of anti-F1 antibodies is useful for serological diagnosis of *Y. pestis*.

In the absence of culture and isolation of *Y. pestis*, plague cases can be confirmed by a 4-fold rise in serum antibody titers against the fraction 1 (F1) antigen of *Y. pestis* by passive hemagglutination testing. Confirmation requires testing of acute and convalescent phase serum samples. Some plague patients develop antibodies within as few as 5 days after the onset of illness, most seroconvert 1 to 2 weeks after onset. Positive serologic titers diminish gradually over months to years.¹⁻⁸

2. IN VITRO ACTIVITY

Levofloxacin has been shown to be active against a wide range of Gram negative isolates including the members of *Enterobacteriaceae* both *in vitro* and in clinical infections. The microbiology subsection of the current levofloxacin package insert does not provide information on the *in vitro* activity of levofloxacin against *Yersinia pestis*.

Several studies have evaluated the *in vitro* activity of levofloxacin against *Y. pestis*. These studies are summarized below.

2.1. Mechanism(s) of action

Fluoroquinolones directly inhibit DNA synthesis through the interaction of the drug complexes with one or both of the target bacterial type II topoisomerase enzymes, DNA gyrase and DNA topoisomerase IV. These enzymes are structurally related, both being heterotetrameric enzyme (A₂B₂) consisting of two different subunit pairs. The GyrA and GyrB subunits of the DNA-gyrase are homologous with the ParC and ParE subunit of the DNA topoisomerase IV, respectively. These bacterial type II topoisomerases play essential roles in bacterial DNA replication, transcription, recombination and repair of DNA.

Fluoroquinolones appear to trap the enzyme on DNA during the topoisomerization reaction, resulting in barrier to the movement of the replication fork. These complexes triggers poorly defined events within the bacterial cell resulting in double-stranded DNA breaks, damage and ultimately cell death.

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2.2. Antimicrobial spectrum of activity

In vitro susceptibility studies of levofloxacin were evaluated against a set of contemporary *Y. pestis* laboratory and clinical isolates. Most of the laboratories used the broth microdilution method in accordance with the Clinical Laboratory and Standards Institute (CLSI) M45-A2 guidelines in testing the *Y. pestis* isolates. Briefly, the broth microdilution method uses standard cation-adjusted Mueller-Hinton Broth (CAMHB) media to a bacterial density of $10^5 - 10^6$ colony forming units (CFU/mL) or equivalent to a 0.5 McFarland standard. Plates were incubated in ambient air at $35 \pm 2^\circ\text{C}$ for 24 hours, with an option for further incubation for 48 hours when growth at 24 hours is insufficient for an endpoint interpretation. Any modifications to the standard broth microdilution methods or special methods used for determination of antimicrobial susceptibility testing were described. Quality control of antibiotic stocks was established by using the *E. coli* ATCC 25922.

2.2.1. National Institute of Health Sponsored Studies

A study by the Health Protection Agency determined the activity of levofloxacin and gentamicin against 13 *Y. pestis* including ATCC strains and outbreak strains from the National Culture Type Collection (NCTC) from different geographical sources. The MICs was performed by the CLSI broth microdilution method (M7-A). The levofloxacin MICs ranged from 0.015 to 0.06 $\mu\text{g/mL}$ (Table 1). The levofloxacin MIC for the CO92 strain, isolated from a patient who contracted pneumonic plague from an infected cat and used in the pivotal African Green monkey (AGM) study is 0.03 $\mu\text{g/mL}$. This was the only isolate tested from the United States; however, the MIC value was similar to the MICs observed for *Y. pestis* isolates sourced from various geographic countries.

Table 1: MIC value range for 13 *Y. pestis* isolates from various geographic sources

Organism	Gentamicin $\mu\text{g/ml}$	Levofloxacin $\mu\text{g/ml}$	Geographical source
<i>Y. pestis</i> CO92	0.5 (n=2)	0.03 (n=3)	USA
<i>Y. pestis</i> NCTC 8775	1- 2	0.03	UK (maybe India)
<i>Y. pestis</i> NCTC 8779	1	0.03 - 0.06	UK (maybe India)
<i>Y. pestis</i> NCTC 5923	0.5- 1	0.03	Java
<i>Y. pestis</i> NCTC 5924	1- 2	0.03 - 0.06	Java
<i>Y. pestis</i> NCTC 2028	1- 4	0.03	Java
<i>Y. pestis</i> NCTC 10029	0.5	0.015 - 0.03	Kenya
<i>Y. pestis</i> NCTC 10030	0.5- 1 (n=3)	0.015 (n=3)	Kenya
<i>Y. pestis</i> NCTC 2868	0.5- 1	0.03- 0.06	India
<i>Y. pestis</i> NCTC 570	0.25- 0.5 (n=3)	0.03 - 0.06 (n=3)	India
<i>Y. pestis</i> NCTC 10330	0.5 (n=3)	0.015 - 0.03 (n=3)	Kenya
<i>Y. pestis</i> NCTC 10329	0.5 (n=3)	0.015 (n=3)	Kenya
<i>E. coli</i> ATCC 25922	1-2	0.015 - 0.06	NA

Source: Study Report #HPA-YpLMIC-2008

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In a study by U.S. Army Medical Research Institute of Infectious Disease (USAMRIID) reported the testing of 30 *Y. pestis* isolates selected based on biovar, IS100 genotype and various geographic regions. The MICs were tested using the broth microdilution method in accordance with CLSI M45-A2 (2010) methods. The levofloxacin MICs ranged from 0.008 to 0.12 µg/mL, with MIC₉₀ values ≤0.06 µg/mL (Table 2).

Table 2: Levofloxacin MICs of 30 *Y. pestis* biovars and ISO100 genotypes from different geographic regions

Organism	MIC (µg/mL)
CO92	0.03
C12	0.03
Antiqua	0.12
Pestoides B	0.03
Pestoides Fmp1	0.03
Yeo154	0.03
Angola	0.015
Java9	0.06
M111(74)	0.06
LaPaz	0.06
195P mp1	0.06
T26 mp3	0.03
KIM 10	0.06
Pest E	0.03
RFPBM 19	0.06
PeXu 429	0.03
Yokohama	0.03
Nicholisk 41	0.015
Nairobi	0.03
South Park	0.06
Cambodia	0.06
27	0.015
31	0.03
390	0.06
590	0.06
25	0.06
316	0.03
366	0.03
Harbin 35	0.008
Pest C	0.015
QC <i>E. coli</i> ATCC25922	0.015

Source: Study Report #RIID-YpLMIC-2005

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2.2.2. Published Studies

Frean *et al.*, (1996) evaluated the activity of levofloxacin against 100 *Y. pestis* clinical isolates in patients with plague from South Africa for the period from 1982 to 1991. The MICs were determined by the agar-dilution method in accordance with CLSI M7-A5 guidelines. The MIC₉₀ values were ≤ 0.03 $\mu\text{g/mL}$ (Table 3).

Table 3: Activity of levofloxacin and comparators against 100 clinical isolates of *Y. pestis* from South Africa

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^a TMP-SMX, trimethoprim-sulfamethoxazole.

Source: Frean *et al.*, (1996) *Antimicrobial Agents and Chemotherapy*

Lonsway *et al.*, (2011) evaluated 26 *Y. pestis* strains from the Centers for Disease Control and Prevention (CDC) and USAMRIID collections. The isolates also included 6 strains that were biovar Antiqua, 7 were biovar Medievalis, 12 were biovar Orientalis and 1 isolate could not be assigned to any biovar. The MICs were determined using the broth microdilution method in accordance with CLSI M2-A7 (2006) guidelines. The levofloxacin MIC₉₀ values were ≤ 0.06 $\mu\text{g/mL}$ which was 2-fold dilution lower than ciprofloxacin; however, levofloxacin was lower than other comparators such as doxycycline, streptomycin and chloramphenicol.

Table 4: Activity of levofloxacin against 26 *Y. pestis* biovars

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Source: Lonsway *et al.*, (2011) *J. Clin. Microb.*

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Ryzhko *et al.*, (2009) evaluated 20 encapsulated strains (FI+) and 20 non-encapsulated strains including isogenic strains *Y. pestis* 231 and *Y. pestis* 231 FI-. The method used for testing the MIC is unknown. The authors stated that the levofloxacin MIC values range from 0.01 to 0.02 µg/mL which was similar to that of other fluoroquinolones ciprofloxacin (0.01 to 0.02 µg/mL), lomefloxacin (0.01 to 0.02 µg/mL), ofloxacin (0.04 – 0.08 µg/mL), however, was slightly 8- to 16-fold lower than perfloracin and moxifloxacin (0.16 to 0.32 µg/mL).

2.3. Bactericidal Activity

Louie *et al.*, (2007) evaluated the *in vitro* bactericidal activity of levofloxacin and comparators against *Y. pestis* ΔCO92. The *Y. pestis* ΔCO92 is an avirulent mutant of the *Y. pestis* CO92, which lacks a capsule and the pCD1 Plasmid but has *in vitro* growth kinetics similar to those of its wild-type progenitor. MICs performed by the broth macrodilution method as well as the MBCs were determined in accordance with standard methods as described by CLSI (M7-A7). The results showed that levofloxacin MICs were 2-fold lower than MBCs against the *Y. pestis* ΔCO92 (Table 5). The authors stated that the results for broth macrodilution and agar-based MICs were identical (data not provided).

Table 5: Broth macrodilution MIC and MBC for levofloxacin and streptomycin against *Y. pestis* CO92

Drug	MIC (µg/mL)	MBC (µg/mL)
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2.4. Intracellular antimicrobial concentration assessment

There were no studies that evaluated the activity of levofloxacin against *Yersinia* inside cells (e.g. macrophages).

2.5. Development of Resistance and Resistance Mechanisms

Resistance to antibiotics currently recommended for therapy and for prophylaxis of *Y. pestis* infections have been described in the literature.^{21,23} For instance, *Y. pestis* isolates from some African countries have been described as showing tetracycline resistance or reduce susceptibility to streptomycin²¹. A high level of resistance to multiple antibiotics (chloramphenicol, streptomycin, tetracycline, sulfonamides, ampicillin, kanamycin, spectinomycin and minocycline) because of a self-transferable plasmid was reported in Madagascar²³. In a study by Wong *et al.* determined 21% resistance to imipenem against 92 *Y. pestis* isolates collected over a 21-year period in the United States. A search of the literature has not revealed any reports of clinical *Y. pestis* isolates resistant to levofloxacin or other fluoroquinolones. Although not confirmed in scientific literature, Russian scientists have claimed to have bioengineered multi-drug resistant strains of *Y. pestis*, including a strain resistant to fluoroquinolones. There is no known cross-resistance

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between levofloxacin and other classes such as penicillins, cephalosporins, aminoglycosides, macrolides and tetracyclines in which *Y. pestis* resistance have been reported.

Resistance to levofloxacin typically results by either mutation in the genes controlling production of bacterial topoisomerase II enzymes, decreased outer membrane permeability or drug efflux. No specific modifying or degrading enzymes have been found to be a mechanism of bacterial resistance to levofloxacin or other fluoroquinolones.

Enterobacteriaceae resistance to levofloxacin develops slowly by multiple step mutations. The frequency of spontaneous mutations *in vitro* to levofloxacin generally occurs at a frequency between $<10^{-9}$ to 10^{-6} .

Louie *et al.* (2007) evaluated the frequency of spontaneous mutation *in vitro* to levofloxacin against *Y. pestis* Δ CO92. Briefly, colonies were grown to late-logarithmic-phase overnight in MHB incubated at 37°C. Five milliliters of bacterial suspension was cultured on antibiotic-free MHA plates and MHA plates that contained 3X or 5X MIC for levofloxacin. Streptomycin was used as a comparator. The plates were incubated at 37°C for 72 hours. The number of colonies growing on the plates was counted. The spontaneous mutation frequency was calculated as the ratio of resistance mutants on the plate to the number of CFU/mL in the original inoculum. The mutation frequency for streptomycin at 3X and 5X MIC was 1.1×10^{-6} and 9.8×10^{-7} CFU, respectively. The mutation frequency for levofloxacin at 3X and 5X MIC were 1.2×10^{-7} and 7.4×10^{-8} CFU and -7.13 log CFU, respectively. The mutation frequency for *Y. pestis* Δ CO92 is within the range of the mutation frequency reported in the levofloxacin labeling.

2.6. Susceptibility Test Methods

The susceptibility testing methods generated for the *in vitro* susceptibility data were determined by the broth microdilution method in accordance with the current CLSI M45-A3 guidelines. In the recently published guidelines, CLSI has established a susceptible breakpoint of ≤ 0.025 μ g/mL for levofloxacin against *Y. pestis*, based on the broth microdilution method.

2.6.1. Disk Diffusion Techniques

Lonsway *et al.*, (2011) conducted a multicenter study comparing the disk diffusion method with the CLSI reference broth microdilution method for susceptibility testing of *Y. pestis*. A total of 26 *Y. pestis* strains were tested which were obtained from the CDC and USAMRIID collections (see description in *Section 2.2.2.* above). All isolates were tested at 2 different sites. MIC testing was performed by standard CLSI methods for the broth microdilution and disk diffusion. Briefly, for disk diffusion testing, inocula were prepared from 18 to 24 hour aerobic cultures grown on 5% sheep blood agar plates by direct colony suspension method in Mueller-Hinton broth (MHB, Remel, Lenexa, KS) to equal a 0.5

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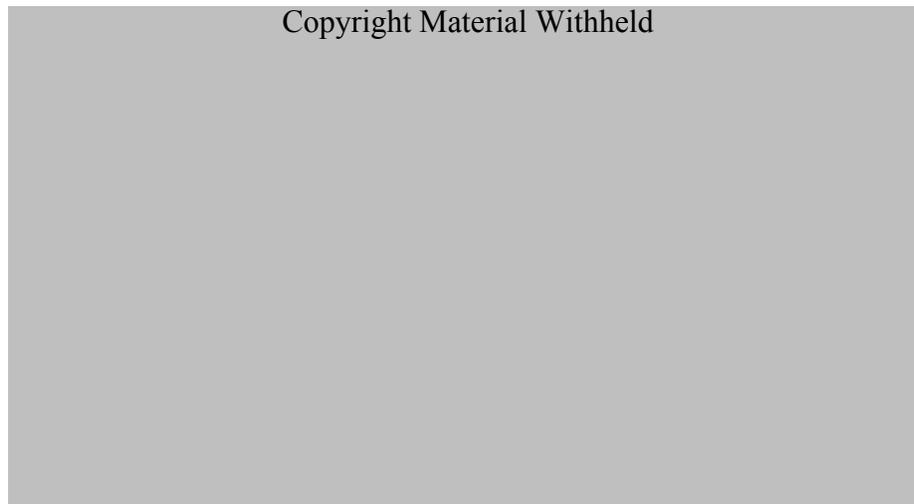
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McFarland turbidity standard. The same inocula were used to inoculate 150-mm diameter Muller-Hinton agar plates. Commercial disks (BBL) were applied with a self-tamping multi-disk dispenser (BML) to plates. The plates and panels for broth microdilution testing were incubated at 35°C and read at 24- and 48-hour time periods. The zone sizes for levofloxacin produced large-diameter zones that ranged from 34 mm to 50 mm (Table 6). Two isolates at one test site were reported to have unreadable zones against all drugs due to poor growth at 24 hours. The authors stated that the zone margins observed for the drugs produced large zones that were often fuzzy or indistinct on the MHA plate and overlapped with other zones of other drugs. It was recommended that the disk diffusion method should not be used to test *Y. pestis* because of the difficulty in reading the poorly defined zones of inhibition and large zone diameters encountered with levofloxacin and other tested antimicrobials.

Table 6: Disk Diffusion results for tests performed using Mueller-Hinton agar against 26 *Y. pestis* isolates at 2 sites



Source: Lonsway *et al.*, (2011) *J. Clin. Microb*

2.6.2. E-test Techniques

Lonsway *et al.*, (2011) also conducted a multicenter study comparing the E-test method with the CLSI reference broth microdilution method for susceptibility testing of *Y. pestis* isolates. Similar to the disk diffusion study, the 26 *Y. pestis* strains obtained from the CDC and USAMRIID collections were tested by the E-test method. All isolates were tested at three different laboratories (A, B and C). Inoculum was prepared similar to the disk diffusion study by making colony suspensions in MHB to equal a 0.5 McFarland turbidity standard. E-test strips were applied to each plate with no more than 4 E-test strips per plate. The plates and panels for broth microdilution testing were incubated at 35°C and read at 24- and 48-hour time periods. The E-test was read at 80% inhibition for the intersection point for chloramphenicol, doxycycline, tetracycline and trimethoprim-sulfamethoxazole in accordance with the E-test package inserts. For levofloxacin, ciprofloxacin, gentamicin and streptomycin were read at 100% inhibition for the intersection point. The E-test MICs

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were rounded up to the nearest \log_2 dilution. The essential agreement for each drug was determined as the percentage of MICs that yielded identical values within $\pm 1 \log_2$ dilution of the broth microdilution method. Comparisons of interpretative category results (susceptible, intermediate and resistant) were performed by calculating the rates of minor, major and very major errors. The MICs for each antimicrobial agent by each method are shown in Table 7. Levofloxacin MICs by the E-test method was similar or within $\pm 1 \log_2$ dilution of the broth microdilution method with 100% category agreement. No errors were observed between the methods.

Table 7: MICs and interpretive category agreement for 48-hour E-test and 48-hour broth microdilution MICs for levofloxacin and comparators against 26 *Y. pestis* isolates tested at 4 sites (104 test results)

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Source: [Lonsway et al., \(2011\) J. Clin. Microb.](#)

Both the broth microdilution and E-test MICs for levofloxacin were $\leq 0.25\mu\text{g/mL}$ after 24 hours of incubation (data not shown) and at 48 hours (Figure 2). Overall, levofloxacin E-test MICs were distributed over 5- \log_2 dilution ranges whereas the broth microdilution encompassed 2- \log_2 dilution ranges (Figure 2 and Table 8).

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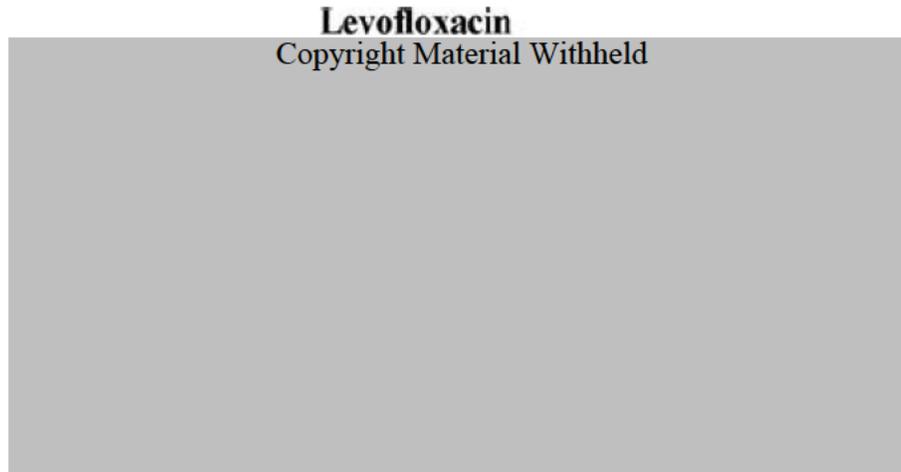
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Figure 2: Distribution of levofloxacin MICs obtained using 48-h E-test and 48-h broth microdilution method against 26 *Y. pestis* isolates tested at 4 sites (n = 104)



Source: Lonsway *et al.*, (2011) *J. Clin. Microb.*

The essential agreement ($\pm 1 \log_2$ dilution) between the two methods was considered to be off the scale for some comparisons since the ranges for the broth microdilution method did not encompass lower concentrations obtainable by the E-test. For instance, some of the isolates had broth microdilution MIC that were $\leq 0.06 \mu\text{g/mL}$ whereas the E-test MIC against these isolates was $0.03 \mu\text{g/mL}$. Generally, the essential agreement for all sites combined (including off-scale MICs) was 100% for levofloxacin (Table 9).

Table 8: Essential agreement between the 48-hour E-test and 48-hour broth microdilution MICs for levofloxacin and comparators against 26 *Y. pestis* isolates tested at 4 sites (n = 104 tests)

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Source: Lonsway *et al.*, (2011) *J. Clin. Microb.*

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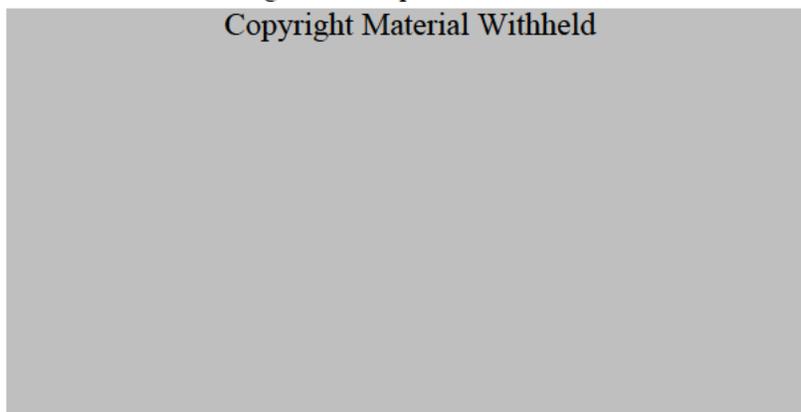
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Table 9: Essential agreement by test site between 48-hour E-test MIC and 48-hour broth microdilution MIC against 26 *Y. pestis* isolates



Source: Lonsway *et al.*, (2011) *J. Clin. Microb.*

3. PHARMACOKINETICS/PHARMACODYNAMICS

3.1. *In vitro*

Louie *et al.*, (2007) simulated human serum concentrations of levofloxacin and comparators *in vitro* and tested against the *Y. pestis* ΔCO92 strain using a hollow-fiber infection model. Briefly, *Y. pestis* ΔCO92 strain was grown to mid-logarithmic phase in MHB. Approximately 10 mL of the *Y. pestis* at a concentration of 10^8 CFU/mL was inoculated into the extra-capillary space of hollow-fiber reactor system (HFS) cartridges incubated at 35°C. Dosing simulations of levofloxacin were tested for the 500 mg dose administered every 24 hours for 10 days. The targeted pharmacokinetic profile for free (non-protein bound) fraction of the dosing regimen of levofloxacin and streptomycin are shown in Table 10. A no treatment hollow-fiber pharmacodynamic model (HFPM) compartment served as a control.

Table 10: Targeted pharmacokinetic-pharmacodynamic parameters for simulation of levofloxacin and streptomycin in a hollow-fiber infection model

Treatment arm	AUC _{24h} (mg.h/L)	C _{max} (mg/L)	Trough (mg/L)	T _½ (h)	Protein binding (%)
Streptomycin, 1 g q12h	--	38	2	2.5	10
Levofloxacin, 500 mg q24h	36	3.6	--	7	30

The MICs for the antibiotics are shown in Table 5.

Bacterial samples (0.6mL) were collected from the HFPM compartments during simulation over the 10 days and quantified on media. Untreated bacteria grew from 10^7 to 10^{10} CFU/mL over 10 days (Figure 3). In the absence of antibiotics (i.e., control untreated HFPM), subpopulations of streptomycin and levofloxacin resistant mutants grew in proportion to the total bacterial population (Table 11). Treatment of HFPM compartment

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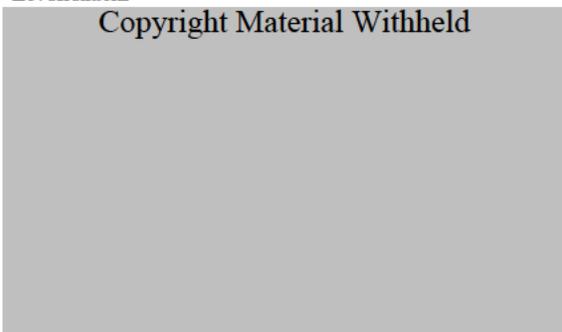
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with streptomycin resulted in a 5-fold \log_{10} decrease in the total bacterial population within 24 hours; however re-growth of organisms were observed with the total bacterial density similar to the control arm by day 7. In contrast, treatment of HFPM compartment with 500 mg q24h levofloxacin resulted in the elimination of organisms below the limit of detection ($<10^2$ CFU/mL). There was a single increase in bacterial counts in the levofloxacin arm on day 6 of the 10-day treatment course. However, at the end of the 10-day study, cultures were below detectable limits in the levofloxacin treatment arm.

Figure 3: Antimicrobial effect of simulated clinical regimens for levofloxacin and streptomycin against *Y. pestis* ΔCO92 in an in vitro hollow fiber infection model

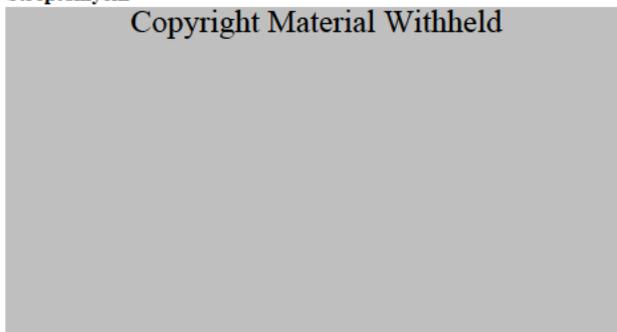
Levofloxacin

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Streptomycin

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Source: Louie *et al.*, (2007) Antimicrob. Agents Chemother.

MICs were determined on days 0, 4, 6 and 10 of the bacterial populations and were assessed for resistance amplification. Organisms were tested from the control, levofloxacin-treated and streptomycin-treated HFPM compartments. Bacterial counts were determined on antibiotic free agar and agar supplemented with 3x MIC of the levofloxacin or streptomycin. The MIC was determined by the macrobroth method with Mueller Hinton Broth and agar dilution method. From the control arm (i.e. no treatment), subpopulations of organisms resistant to streptomycin (6 – 8 $\mu\text{g/mL}$) and levofloxacin (0.25 – 1 $\mu\text{g/mL}$) grew in proportion to the total bacterial population (Table 11). Re-growth in the streptomycin-treated HFPM compartment was due to organisms that were resistant to streptomycin. During the first days of therapy, the authors noted a mixture of small-colony variants with MICs equivalent to that of the parent strain (2 $\mu\text{g/mL}$) and larger colonies with increased MICs were cultured from streptomycin-supplemented agar. Streptomycin MICs increased with the duration of streptomycin therapy. In the levofloxacin treatment arm, mutants with increased MICs to the drug were isolated on antibiotic supplemented agar only prior to the initiation of therapy on day 0. Though there was a single increase in bacterial counts in the levofloxacin arm on day 6 of the 10-day treatment course, the levofloxacin MICs against these isolates were <1 $\mu\text{g/mL}$.

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Table 11: *In vitro* MIC of *Y. pestis* resistant mutants selected on agar plates supplemented with 3X MIC of levofloxacin or streptomycin over the 10-day study

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Source: Louie *et al.*, (2007) *Antimicrob. Agents Chemother.*

The growth fitness of the streptomycin and levofloxacin mutants from the above experiment were evaluated in both immune normal and neutropenic mouse thigh infection models. The mouse thigh infection model was used for the growth fitness studies because preliminary experiment showed that the parent *Y. pestis* Δ CO92 strain does not grow in the spleens of immune normal and neutropenic mice. Briefly, female Swiss Webster mice were rendered neutropenic by the administration of 150 mg/kg cyclophosphamide given intraperitoneally (i.p.) 3 days prior to bacterial inoculation followed by 100 mg/kg i.p. dose of cyclophosphamide every other day. This regimen resulted in persistent neutropenia beginning on the day the second dose of cyclophosphamide was administered. Groups of neutropenic and immune normal mice (21/group) were injected in each posterior thigh muscle with 10^6 CFU of the pan-susceptible *Y. pestis* parent Δ CO92 strain, 10 *Y. pestis* strains with increased MICs to levofloxacin (1 μ g/mL) and 10 streptomycin-resistant isolates (8 μ g/mL). Five mice per group were sacrificed on days 0, 1, 2, 3, 4, 5 and 7. The infected thigh muscles were aseptically collected, homogenized and quantitatively cultured. The plates were incubated at 35°C for 96 hours. The MICs were determined for 10 colonies isolated from the muscle of homogenates each day to confirm that the susceptibility profile had not changed over the 7-day period. Overall, the growth of the 10 streptomycin resistant mutants was similar to the growth of parent *Y. pestis* Δ CO92 strain in both the neutropenic and immune normal thigh infection models. In contrast, 9 of the 10 levofloxacin isolates were less fit than the parent *Y. pestis* Δ CO92 strain. The growth curves for the parent strain, 2 of the streptomycin resistant mutants and 3 of the 10 levofloxacin resistant mutants in the neutropenic and immune normal mice are represented in Figure 4. The growth curves for 8 streptomycin resistant isolates not shown in the Figure were similar to that parent strain. Similarly, the growth curves for the 7 levofloxacin-resistant mutants were similar to those of the less fit mutants shown in the Figure. Susceptibility studies conducted on isolates cultured from homogenates of thigh muscles over the 7-day studies demonstrated that the MICs had not changed over time.

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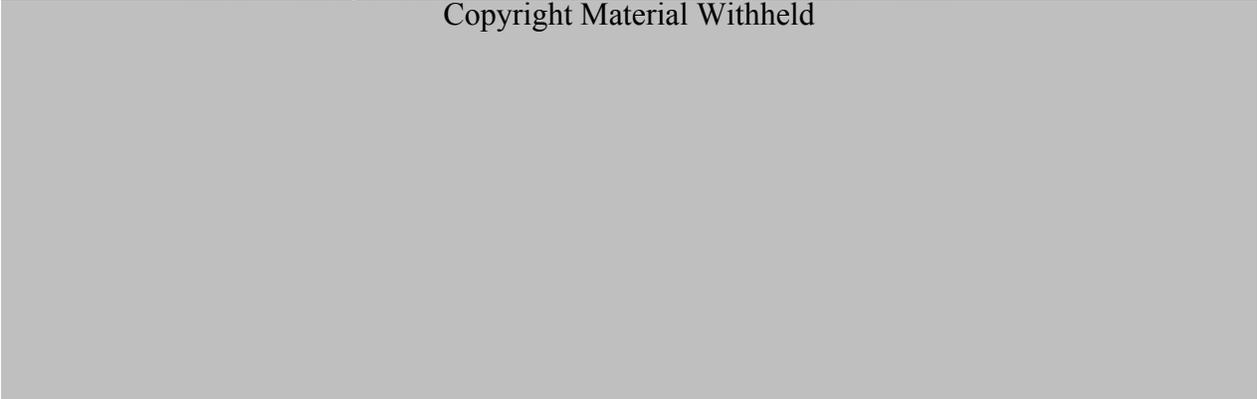
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Figure 4: Growth fitness of parent *Y. pestis* ΔCO92 strain and streptomycin- and levofloxacin-resistant mutants in neutropenic and immune normal mouse thigh infection models

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Source: [Louie et al., \(2007\) Antimicrob. Agents Chemother.](#)

3.2. *In vivo*

Pharmacokinetic studies in healthy AGMs administered single or repeat doses of levofloxacin was shown to be rapidly absorbed after oral administration, with C_{max} and AUC values noted to increase in a dose-dependent or dose proportional manner (Table 12). There were no significant differences observed in the PK parameters in monkeys given single or repeated IV levofloxacin doses. The mean terminal plasma elimination half-life ($t_{1/2}$) was short (approximately 3 hours) and the oral bioavailability was high (80%).

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Table 12: Mean pharmacokinetic parameters following single or repeat doses of levofloxacin in African Green monkeys

Study Type/ (Study No.)	N	Route	Duration	Dose (mg/kg)	C _{max} (µg/mL)	t _{max} (h)	Vz or		t _{1/2} (h)	Cl or CL/F (mL/h/kg)	F (%)
							Vz/F (mL/kg)	AUC _(0-∞) (µg·h/mL)			
Single and Repeat-Dose PK (Study B122-03) ^b	3/sex ^b	p.o.	once	15	5.00 (3.24)	1.50 (0.77)	3317.48 (947.27)	24.16 ^d (5.99)	4.74 (1.75)	504.32 (92.48)	80
			(SDE)	20	5.22 (1.65)	1.50 (0.77)	4084.37 (540.23)	32.56 (5.74)	5.77 (1.19)	503.54 (88.46)	80
		once	25	5.82 (1.26)	2.60 (1.33)	3145.57 (509.47)	36.01 (6.17)	4.24 (1.09)	528.38 (78.80)	74	
	(20-min inf)	IV	once	15	13.36 (2.86)	0.33 (0.00)	2334.88 (216.48)	30.55 (5.30)	3.30 (0.65)	503.89 (90.19)	NA
			14 days (1 st dose) ^c	20	16.92 (1.48)	0.30 (0.07)	2570.44 (125.68)	45.35 (8.23)	3.21 (0.44)	566.99 (106.04)	NA
			14 days (last dose) ^c	20	11.83 (1.78)	0.25 (0.09)	3036.95 (521.34)	30.44 (4.26)	2.52 (0.31)	836.34 (130.07)	NA
			Once	8	3.17 (0.24)	0.09 (0.01)	3559 (729)	8.86 (2.01)	2.65 (0.15)	932 (191)	NA
Single Dose PK (Study FY08-150) ^f	3 F	IV (30-min inf)	Once	2	0.71 (0.60)	12.87 (0.24)	4167 (NC) ^e	3.58 (NC) ^e	5.17 (NC) ^e	582 (NC) ^e	NA
			Once	8	3.25 (0.36)	0.60 (0.01)	1980 (300)	10.30 ^g (2.33)	2.36 (0.44)	602.15 (179.28)	NA
Single Dose PK (Study B465-10) ^f	3M	IV (30-min inf)	Once	8	3.34 (0.21)	0.60 (0.01)	2300 (110)	7.95 ^g (0.69)	2.01 (0.01)	793.83 (37.93)	NA
			Once	2	0.90 (0.22)	12.80 (0.1)	2500 (710)	3.53 ^h (1.33)	2.81 (0.61)	661.62 (340.76)	NA
	3 F	IV (30-min inf)	Once	2	0.72 (0.10)	12.60 (0.01)	3600 (690)	2.04 ^h (0.32)	2.57 (0.17)	964.32 (130.89)	NA
			Once	8	3.17 (0.24)	0.09 (0.01)	3559 (729)	8.86 (2.01)	2.65 (0.15)	932 (191)	NA

^a Values in parentheses are standard deviation.

^b Study B122-03 consisted of 3 phases; monkeys (3/sex) were re-used at each dose and study phase. In Phase I, monkeys received single escalating p.o. (nasogastric) doses of 15, 20, and 25 mg/kg on Days 1, 15 and 29, respectively, with a 2-week washout period between doses. In Phase II, monkeys received a single 20-minute IV infusion of 15 mg/kg on Day 43, and in Phase III, monkeys received repeated 20-minute IV infusions of 20 mg/kg for 14 days, from Day 85 to Day 98.

^c PK values are shown for Phase III of Study B122-03 for the first (Day 85) and last (Day 98) of 14 days of IV doses. Values shown are for total drug.

^d AUC_{0-8h}

^e N=2.

^f Monkeys (3 F) in Study FY08-150 and in Study B465-10 (3/sex) were administered a 30 minute IV infusion of 8 mg/kg levofloxacin followed by a dose of 2 mg/kg 12 hours later.

^g AUC_{0-12h}

^h AUC_{12-24h}

F = female; h = hours; inf. = infusion; IV = intravenous; min = minutes; N = number; NA = not applicable;

NC = not calculable; No. = number; p.o. = oral; SDE = single dose escalation

The PK profiles of humanized IV dosing regimen of 8 mg/kg high dose followed by a second infusion within 12 hours of 2 mg/kg resulted in a C_{max} and AUC_{0-24h} values of 3.3 µg/mL and 11.4 µg.h/mL respectively (Table 13). The PK values in AGMs were noted to be significantly lower than those in adult humans administered 500 mg IV once daily (6.2 – 6.4 µg/mL and 48.3 – 54.6 µg.h/mL, respectively). However, throughout the 24-hour dosing period, the plasma concentrations of levofloxacin in AGMs did not exceed that observed in an adult human administered 500 mg IV or oral once daily dosing (Figure 5).

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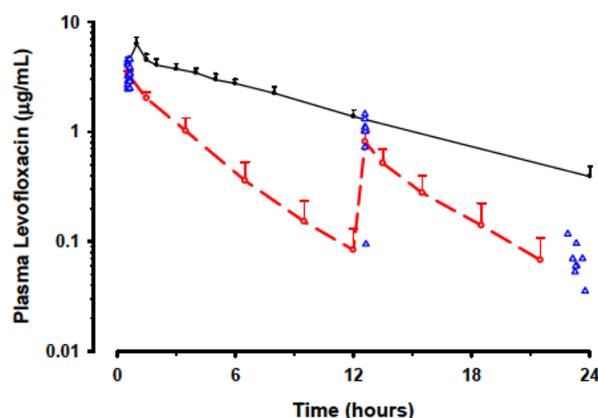
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Table 13: Comparison of levofloxacin pharmacokinetic parameters in adult human and African Green monkeys

Species	IV Dose	AUC/MIC ₁₀₀ Ratio ^a	C _{max} (µg/mL)	% Human C _{max} (500 mg)	AUC _{0-24h} (µg·h/mL)	% Human AUC (500 mg)	Trough Conc. (µg/mL)
Adult Human Volunteer ^d	500 mg IV QD Single	402	6.2	NA	48.3	NA	0.5
Adult Human Volunteer ^d	500 mg IV QD Multiple	455	6.4	NA	54.6	NA	0.6
African Green Monkey (Study B465-10)	8/2 mg/kg IV ^b Single	99	3.3	53	11.9	25%	<0.03-0.06 ^c

Figure 5 : Levofloxacin plasma concentrations following intravenous (IV) dosing in AGMs and humans.



The solid line and the closed circles represent concentrations in humans following a single IV administration of levofloxacin 500 mg (Study LOFBO-PHIO-097, n=23). The dashed line and the open circles represent concentrations in healthy AGM following a single administration of levofloxacin 8/2 mg/kg (Study B465-10, n=6). The open triangles represent peak and trough concentrations in diseased AGM following multiple IV administrations of levofloxacin 8/2 mg/kg (Cohort 3 of Study FY07-070).

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4. ANIMAL MODELS OF INFECTION

Several studies evaluated the activity of prophylactic and therapeutic levofloxacin in animal models of experimental pneumonic plague.

4.1. Mice

The experimental procedures were comparable across studies. Briefly, mice were exposed to *Y. pestis* organisms by nasal installation, using a nose-only or whole body aerosol system. All studies tested the *Y. pestis* CO92 strain which was isolated from a fatal human case of pneumonic plague that was exposed to an infected cat. Dosing and schedule under normal and neutropenic conditions were tested. Animals were exposed to *Y. pestis* CO92 strain at concentrations that ranged from 1.7×10^3 CFU/mL to 6.84×10^4 CFU/mL. Treatment with levofloxacin was initiated at 24- or 36-hours (post-exposure) or at 42- or 48-hours (treatment) post-challenge. Treatment was for 5 or 7 days. Efficacy was determined by observing the number of surviving mice post-challenge. Control animals died of pneumonic plague 3 – 4 days post-challenge; however, few animals show any outward signs of disease. Symptoms when they occur appear 40 – 45 hours after exposure.

Levofloxacin was equally effective as ciprofloxacin and provided complete protection in the post-exposure (24-hours post-challenge) model (Table 14). Dosing as low as 1.5 mg/kg/day when given twice daily was protective, with an ED₅₀ of 0.7 mg/kg/day. It was observed that neutropenia had no effect on levofloxacin efficacy in the post-exposure model. Upon re-challenged with *Y. pestis*, the majority of the levofloxacin mice succumbed to infection. Antibody titers were not performed in animals. Overall, complete protection was achieved with doses of levofloxacin equal to or greater than 5 mg/kg/day.

Table 14: Activity of levofloxacin and comparators prophylaxis treatment against *Y. pestis* CO92 strain in an experimental pneumonic plague murine model

Start of Prophylaxis treatment	Challenge Dose	MIC (µg/mL)	Compound	Dose (mg/kg)	Dosing Frequency	Survival (%)	Time to Death	Reference
24-hours post challenge	1.05 x 10 ⁴ (challenge)	0.03	Levofloxacin	25	i.p. q24h 6d	90	20d	UTMB- YpEff-1-8
		0.03	Levofloxacin	50		100	--	
		0.03	Levofloxacin	100		100	--	
		0.03	Levofloxacin	150		90	20d	
		--	Control	0		0	3d	
	2.1 x 10 ⁴ (re-challenge at Day 21)	0.03	Levofloxacin	25	NT	0	23d	
		0.03	Levofloxacin	50		20%	23 – 26d	
		0.03	Levofloxacin	100		10%	23 – 26d	
0.03		Levofloxacin	150	0		23 – 26d		
24-hours post challenge	1.05 x 10 ⁴ (challenge)	0.03	Levofloxacin	0.01	i.p. q24h 6d	0	4d	UTMB- YpEff-1-8
		0.03	Levofloxacin	0.05		0	4d	
		0.03	Levofloxacin	0.1		0	4d	
		0.03	Levofloxacin	0.5		22	5 – 14d	

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Start of Prophylaxis treatment	Challenge Dose	MIC (µg/mL)	Compound	Dose (mg/kg)	Dosing Frequency	Survival (%)	Time to Death	Reference
		0.03	Levofloxacin	1		80	8 – 15d	
		0.03	Levofloxacin	5		90	13 – 14d	
		0.03	Levofloxacin	10		100	--	
		0.03	Levofloxacin	15		100	--	
		--	Control	0		0	4d	
	1.05 x 10 ⁴ (re-challenge on day 29)	0.03	Levofloxacin	0.5	NT	0	34d	UTMB-YpEff-1-8
		0.03	Levofloxacin	1		30	34d	
		0.03	Levofloxacin	5		40	34 – 36d	
		0.03	Levofloxacin	10		50	35 – 43d	
		0.03	Levofloxacin	15		10	34d	
		--	Control	0		0		
	1.05 x 10 ⁵ (re-challenge on day 74)	0.03	Levofloxacin	1	NT	10	76d	UTMB-YpEff-1-8
		0.03	Levofloxacin	5		20	80 – 85d	
		0.03	Levofloxacin	10		20	76 – 83d	
		0.03	Levofloxacin	15		0	0	
24-hours post challenge	1.05 x 10 ⁴ (challenge)	0.03	Levofloxacin	0.1	i.p. q24h 6d	0	4 d	UTMB-YpEff-1-8
		0.03	Levofloxacin	0.5		0	4 – 7d	
		0.03	Levofloxacin	1		80	6 – 15d	
		0.03	Levofloxacin	5		100	--	
		0.03	Levofloxacin	10		100	--	
		0.03	Levofloxacin	15		100	--	
	--	Control	0	0	4d			
	1.05 x 10 ⁴ (re-challenge on day 34)	0.03	Levofloxacin	1	NT	20	36 – 40d	UTMB-YpEff-1-8
		0.03	Levofloxacin	5		10	36 – 40d	
		0.03	Levofloxacin	10		20	36 – 40d	
0.03		Levofloxacin	15	20		36 – 42d		
24 hours post-challenge	1.05 x 10 ⁴ (challenge)	0.03	Levofloxacin	5	i.p. q24h 6d	100	--	UTMB-YpEff-1-8
		0.03	Levofloxacin	10		100	--	
		--	Control	0		0	3d	
24-hours post challenge	6.8 x 10 ⁴	0.03	Levofloxacin	1.5	i.p. q12h 5d	100	--	RIID-YpEff-2006
		0.03	Levofloxacin	3		100	--	
		0.03	Levofloxacin	7.5		100	--	
		0.03	Levofloxacin	15		90	32d	
		--	Control	0		0	3 – 4d	
24-hours post challenge	6.8 x 10 ⁴	0.03	Levofloxacin	15	i.p. q12h 5d	100	--	RIID-YpEff-2006
		--	Ciprofloxacin	30	i.p. q12h 5d	100	--	
		--	Gentamicin	12	i.p. q6h 5d	100	--	
		--	Control	0	--	0	3 – 4d	
24-hours post	6.8 x 10 ⁴ normal mice	0.03	Levofloxacin	15	i.p. q12h 5d	100	--	RIID-YpEff-
		--	Gentamicin	30	i.p. q6h 5d	90	15d	

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Start of Prophylaxis treatment	Challenge Dose	MIC (µg/mL)	Compound	Dose (mg/kg)	Dosing Frequency	Survival (%)	Time to Death	Reference
challenge	6.8 x 10 ⁴ neutropenic mice	0.03	Levofloxacin	15	i.p. q12h 5d	100	--	2006
		--	Gentamicin	12	i.p. q6h 5	70	7 – 20d	
		--	Control	0	--	0	3 – 4d	
24-hours post challenge	2.3 x 10 ⁴ normal mice	0.03	Levofloxacin	15	i.p. q12h 5d	100	--	Heine <i>et al.</i> , (2007)
		0.5	Doxycycline	40	q6h 5d	90	13d	
		0.5	Gentamicin	12	q6h 5d	90	15d	
		0.5	Gentamicin	24	q12h 5d	80	10 – 15d	
		0.5	Gentamicin	48	q24h 5d	80	10 – 13d	
		0.5	Control	0	--	0	3 – 4d	
	2.3 x 10 ⁴ neutropenic mice	0.03	Levofloxacin	15	i.p. q12h 5d	100	--	
		0.5	Doxycycline	40	q6h 5d	0	9 - 13d	
		0.5	Gentamicin	12	q6h 5d	70	9 – 20d	
		0.5	Gentamicin	24	q12h 5d	100	--	
		0.5	Gentamicin	48	q24h 5d	80	15 – 20d	
		0.5	Control	0	--	0	3 – 4d	
24-hours post challenge	1.7 x 10 ³	0.03	Levofloxacin	0.1	i.p. q24h 6d	0	4d	Peterson <i>et al.</i> , (2010)
		0.03	Levofloxacin	0.5		0	4 – 7d	
		0.03	Levofloxacin	1		80	6 – 16d	
		0.03	Levofloxacin	5		100	--	
		0.03	Levofloxacin	10		100	--	
		0.03	Levofloxacin	15		100	--	
		--	Control	0		0	3 – 4d	
		--	Control	0		0	3 – 4d	
24-hours post-challenge	1.7 x 10 ³	0.03	Levofloxacin	5	i.p. q24h 6d	100	--	Peterson <i>et al.</i> , (2010)
		0.03	Levofloxacin	10		100	--	
		--	Control	0		0	3d	

d = days; i.p. = intraperitoneal administration; p.o. = oral administration; s.c = subcutaneous administration, q12h = twice daily; NT = not treated

^aCO92 (CDC) = Original CO92 strain from the Centers for Disease Control and Prevention (CDC)

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When levofloxacin treatment is delayed to 36-hours post-challenge, 90% of mice survived out to 40 days post-challenge for the 5 mg/kg and 10 mg/kg treatment groups (Table 15). Further delay of levofloxacin treatment to 48 hours post-challenge resulted in 20% and 10% survival for the 10 mg/kg/day and 5 mg/kg/day treatment groups, respectively.

Table 15: Activity of levofloxacin and comparators in delayed treatment in an experimental pneumonic plague murine model

Start of Prophylaxis treatment	Challenge Dose	MIC (µg/mL)	Compound	Dose (mg/kg)	Dosing Frequency	Survival (%)	Time to Death	Reference
36hours post-challenge	1.05 x 10 ⁴ (challenge)	0.03	Levofloxacin	5	i.p. q24h 6d	90	8d	UTMB-YpEff-1-8
		0.03	Levofloxacin	10		90	3d	
		--	Control	0		0	3d	
		0.03	Levofloxacin	10		20	6-12d	
		--	Control	0		0	3d	
36hours post-challenge	1.05 x 10 ⁴	0.03	Levofloxacin	5	i.p. q24h 6d	90	8d	Peterson <i>et al.</i> , (2010)
		0.03	Levofloxacin	10		90	3d	
		--	Control	0		0	3d	
42-hours post challenge	6.8 x 10 ⁴	0.03	Levofloxacin	15	i.p. q12h 5d	70	4d	RIID-YpEff-2006
		--	Ciprofloxacin	30	i.p. q12h 5d	50	4d	
		--	Gentamicin	12	i.p. q6h 5d	70	4d	
		--	Control	0	--	0	3-4 d	
48 hours post-challenge	1.05 x 10 ⁴	0.03	Levofloxacin	5	i.p. q24h 6d	10	6-12d	UTMB-YpEff-1-8
		0.03	Levofloxacin	10		20	6-12d	
		--	Control	0		0	3d	
48 hours post-challenge	1.05 x 10 ⁴	0.03	Levofloxacin	5	i.p. q24h 6d	10	6-12d	Peterson <i>et al.</i> , (2010)
		0.03	Levofloxacin	10		20	6-12d	
		--	Control	0		0	3d	

d = days; p.o. = oral administration; s.c = subcutaneous administration, q12h = twice daily

4.2. Rats

In a rat model of infection, groups of rats (weighing 96 – 107 g) were exposed via nasal installation to 6.0 x 10³ CFU of the *Y. pestis* CO92 strain. Different doses (0.5, 1, 5, 10 and 15 mg/kg/day i.p. 6days) of levofloxacin administered at 24 hours post-challenge. Treatment was for 6 days. Efficacy was determined by observing the number of surviving rats 42 days post-challenge. Survivors were re-challenged with 3.5 x 10³ CFU and monitored for an additional 17 days (Day 60 post-challenge). Untreated control animals died 3 to 6 days post-challenge (Table 16). Levofloxacin provided complete protection at doses as low as 5/mg/kg administered once daily. Delayed treatment, no later than 42 hours post-challenge in rats resulted in complete protection. The majority of rats survived upon rechallenge with *Y. pestis*.

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Table 16: Activity of levofloxacin in a rat model of experimental pneumonic plague

Start of Prophylaxis treatment	<i>Y. pestis</i> Strain	Challenge Dose (CFU)	MIC (µg/mL)	Compound	Dose (mg/kg)	Dosing Frequency	Survival (%)	Time to Death	Reference		
24-hours post challenge	CO92 (BEI)	6.0 x 10 ³ (challenge)	0.03	Levofloxacin	0.5	i.p. once daily 6d	0	3 - 4d	UTMB-YpEff-1-8		
			0.03	Levofloxacin	1		17	3 - 4d			
			0.03	Levofloxacin	5		67	7 - 11d			
			0.03	Levofloxacin	10		100	--			
			0.03	Levofloxacin	15		100	--			
			--	Control	0		0	3 - 4d			
				3.5 x 10 ³ (re-challenge on Day43 post-challenge)	0.03	Levofloxacin	1		17	--	UTMB-YpEff-1-8
					0.03	Levofloxacin	5		67	--	
					0.03	Levofloxacin	10		100	--	
					0.03	Levofloxacin	15		67	46d	
24-hours post-challenge	CO92 (BEI)	7.0 x 10 ³ (challenge)	0.03	Levofloxacin	5	i.p. once daily 6d	100	--	UTMB-YpEff-1-8		
			0.03	Levofloxacin	10		100	--			
			--	Control	0		0	4 - 6d			
		6.0 x 10 ³ (re-challenge Day 34)	0.03	Levofloxacin	5	NT	100	--			
			0.03	Levofloxacin	10		100	--			
36-hours post-challenge	CO92 (BEI)	7.0 x 10 ³ (challenge)	0.03	Levofloxacin	5	i.p. once daily 6d	100	--	UTMB-YpEff-1-8		
			0.03	Levofloxacin	10		100	--			
			--	Control	0		0	4 - 6d			
		6.0 x 10 ³ (re-challenge Day 34)	0.03	Levofloxacin	5	NT	86	38d			
			0.03	Levofloxacin	10		86	38d			
42-hours post-challenge	CO92 (BEI)	7.0 x 10 ³ (challenge)	0.03	Levofloxacin	5	i.p. once daily 6d	100	--	UTMB-YpEff-1-8		
			0.03	Levofloxacin	10		100	--			
			--	Control	0		0	4 - 6d			
		6.0 x 10 ³ (re-challenge Day 34)	0.03	Levofloxacin	5	NT	89	37d			
			0.03	Levofloxacin	10		89	37d			
48-hours post-challenge	CO92 (BEI)	7.0 x 10 ³ (challenge)	0.03	Levofloxacin	5	i.p. once daily 6d	44	5 - 7d	UTMB-YpEff-1-8		
			0.03	Levofloxacin	10		44	5 - 6d			
			--	Control	0		0	4 - 6d			
		6.0 x 10 ³ (re-challenge Day 34)	0.03	Levofloxacin	5	NT	33	37d			
			0.03	Levofloxacin	10		44	--			
24-hours post-challenge	CO92 (BEI)	1.2 x 10 ⁴ (challenge)	0.03	Levofloxacin	20	Oral 6d	100	--	UTMB-YpEff-1-8		
			0.03	Levofloxacin	20	i.p. 6d	100	--			
			--	Control	0	Oral 6d	20	4 - 6d			

4.3. African Green monkeys

Studies were conducted in African Green monkeys (AGM, *Chlorocebus aethiops* formerly *Cercopithecus aethiops*) as an experimental model to be used under the Animal Rule (21 CFR 314.610) to support a treatment indication for inhalational plague in adults and pediatric patients (>50 kg and ≥ 6 months of age).

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4.3.1. Determination of Median Lethal Dose of *Y. pestis* CO92 strain

A single study (#D93-16) conducted at USAMRIID was performed to assess the median lethal dose (LD₅₀) in which 50% of AGMs succumbed to infection to aerosolized *Y. pestis* CO92 strain. This study served as the basis for subsequent natural history studies of inhalational plague in AGMs. This study was reviewed previously for details see *IND 64,429 Clinical Microbiology Review* dated 10/12/2011.

Briefly, groups of animals (4 per group) received approximately 10⁴, 10³, 10² or 10¹ CFU by head-only aerosol exposure to *Y. pestis* CO92 strain. The range of aerosols in these 16 animals was 14 to 27,550 CFUs (Table 17). Nine animals succumbed to the infection (range, 315 – 27,550 CFUs) and seven animals survived the challenge (range, 14 – 1,803 CFUs). None of the animals were euthanized. Time to death ranged from 4 to 9 days with an average of 6.3 days.

Table 17: Summary of lethal outcome of the 16 AGMs challenged to *Y. pestis* via aerosol route

AGM#	Sex	Weight (kg)	Estimated Inhaled Dose (CFU)	Outcome	Time of Death
02465	Male	6.4	27,550	Died	Day 7
02456	Female	4.5	1,775	Died	Day 5
02459	Female	4.43	315	Died	Day 5
8978	Male	5.2	17	Survived	
133K	Female	*	18	Survived	
9204	Female	3.9	272	Survived	
T646	Male	6.4	1,803	Survived	
T603	Male	4.3	1,380	Died	Day 9
153K	Female	3.7	1,000	Died	Day 7
T777	Male	5.8	140	Died	Day 9
T807	Male	5.7	157	Survived	
T627	Male	5.4	15	Survived	
1210	Female	5.6	14	Survived	
T775	Female	3.4	9,967	Died	Day 4
T344	Female	4.3	13,050	Died	Day 4
T735	Male	4.6	22,750	Died	Day 7

*Unknown weight

Gross necropsies were performed on the 9 monkeys that succumbed to the challenge. All 9 animals had primary pneumonic plague with secondary bacteremia as the cause of death. Gross pathologic findings of the lesions in the lungs were reported to have varying degrees of edema, acute hemorrhage, acute inflammation and had mild to severe intra-alveolar flooding that sometimes involved both lungs (#02465, T775, 02456, T603, T777) and being marked in one lung and moderate (#153K, 02459) or minimal (#T344, T735) in the

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other lung. All animals had serosanguinous exudates in the pleural cavities and red frothy material in nares. The infection and inflammation had spread to the mediastinal lymph node and adjacent mediastinum. Histologically, in the bone marrow, there was an increased ratio of proliferating to non-proliferating pools of the myeloid series cells, also an increase in the number of intravascular marginated neutrophils, indicating an increase in the circulating and/or marginated pool of neutrophils. Lesions were also noted to be mild to moderate in the pleural cavity, lymph node, spleen, liver and in some animal's larynx, trachea and brain/meninges. The lesions were characterized as moderate to mild and contained bacteria that by microscopic examination were consistent with *Y. pestis* morphology. Cultures of the tissue were not done. One animal (#T777) had fibrin thrombi in the renal glomeruli and hemorrhage in the stomach wall suggestive of disseminated intravascular coagulation (DIC).

4.3.2. Natural History Studies

Several laboratories studied the natural progression of pneumonic plague in AGMs after experimental aerosol exposure to the *Y. pestis* CO92. All studies were funded through the National Institute of Allergy and Infectious Diseases (NIAID) in the Division of Microbiology and Infectious Diseases (DMID). All studies used AGMs, *Y. pestis* CO92, aerosol challenge route, target challenge dose and recorded the same parameters. The objectives were to determine the disease progression of untreated pneumonic plague in AGMs infected with *Y. pestis* CO92 which includes the time of onset of fever, respiratory rate, heart rate, clinical signs and symptoms, bacteremia and pathologic findings. Progression of disease in AGMs was compared with that in humans to identify the parameter(s) that would be optimal as a trigger for initiation of therapeutic intervention in efficacy studies conducted with AGMs.

These natural history studies were previously reviewed. For details see *IND 64,429 Clinical Microbiology Review* dated 7/12/2011 and 10/20/2011. The results are summarized below.

Animals

A total of 36 AGMs (20 females and 16 males) were studied at the following facilities:

- The United States Army Medical Research Institute of Infectious Diseases (USAMRIID, Fort Detrick, Frederick MD) performed a study in 6 AGMs; 3 males and 3 females (#F03-09G).
- The Lovelace Respiratory Research Institute conducted a study in April 2007 which included 5 males and 5 females AGMs (#FY06-126).
- Two studies were conducted at the Battelle Biomedical Research Center (BBRC; West Jefferson, OH):
 - Study #617-G607610 was conducted in July 2007 that included 10 AGMs (3 male and 7 females)

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- Study #875-G607610 was conducted in January 2009 that included 10 AGMs (5 males and 5 females).

All studies used the *Chlorocebus aethiops* formerly *Cercopithecus aethiops*, also known as African Green monkeys (AGMs), which were wild caught from a colony on the island of St. Kitts West Indies. No natural risk of plague has been reported on the island of St. Kitts which is considered plague-free (communication from the *Centers of Disease Control and Prevention*). The National Institute of Health (NIH, Apha Genesis, Yemassee SC) provided the animals to each laboratory. All animals were adults, weighing approximately 3.0 to 6.0 kg. Each facility examined and performed health screens at the time of quarantine and prior to placement in the study. However, individual health screens performed on animals were examined differently by each laboratory. Overall, all animals used in the in-life phase were deemed of acceptable health and free of malformations by a qualified veterinarian. The criteria used to define acceptable health were not specified nor standardized across the facilities.

Prior to placement in the in-life phase of the study, each AGM was implanted with a telemetry monitoring device. Though the monitoring devices differed by laboratory site, each device was capable of measuring and recording body temperature. The transmitters also measured respiratory rate, heart rates, pulse rate, blood pressure, electrocardiography activity and cardiovascular function; however, these measurements varied by laboratory and were not standardized across sites. All monkeys were maintained in individual stainless steel cages and were provided water *ad libitum*. Fresh feed was provided at least twice daily.

Challenge organism

The challenge strain used was the *Y. pestis* CO92 biovar Orientalis. The *Y. pestis* CO92 originated from the same source, the Centers for Disease Control and Prevention (CDC) (Figure 6). There was no attempt to control strain passages across studies. Battelle and Lovelace obtained the CO92 strain from 2nd and 3rd sources through the University of Chicago or University of New Mexico, respectively.

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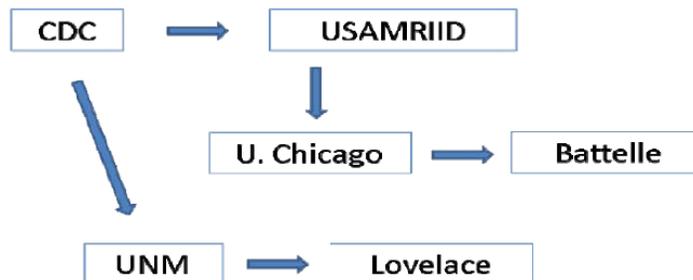
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Figure 6: Sources of *Y. pestis* CO92 strain used in the Natural History studies



Battelle = Battelle Biomedical Research Center; CDC = United States Centers for Disease Control and Prevention; Lovelace = Lovelace Respiratory Research Institute; U. Chicago = University of Chicago; UNM = University of New Mexico; USAMRIID = United States Army Medical Research Institute of Infectious Diseases.

Characterization studies of the master and working stocks of *Y. pestis* CO92 were performed at Lovelace (FY06-126) which confirmed the cultures were pure *Y. pestis* free of other microbes and fungi. In addition, Lovelace performed Gram stain and examined colony characteristics on different media to show that the organisms cultured were consistent with *Y. pestis*. Molecular and animal studies were also conducted to confirm that the *Y. pestis* master and working stocks contain the pCD1, pMT1 and pPla plasmids and were pathogenic in Swiss Webster Mice. No characterization studies of the CO92 strain were provided by the Battelle and USAMRIID laboratories. It is unknown if the differences in maintenance and culture conditions could be responsible for the variability observed in the studies.

Aerosol Preparation

USAMRIID prepared the inoculum in brain heart infusion broth whereas Battelle prepared the inoculum in sterile phosphate buffered saline + 0.01% gelatin containing 9.7% (wt/vol) trehalose. Lovelace did not provide the methods used to prepare aerosol suspensions for challenge. The starting concentration of the nebulizers were either estimated or quantified depending on the study. However, the starting concentrations ranged from 3×10^6 CFU/mL to 8.08×10^8 CFU/mL. Battelle study #617-G607610 had starting nebulizer concentrations that were more than a log-fold higher (10^8 CFU/mL) than other studies ($10^6 - 10^7$ CFU). This difference accounted for a significant animal to animal variability in challenge dose compared to the other studies. This study (#617-G607610) was repeated after additional work, by study# 875-G607610. However, study #617-G607610 was included in support of the general disease pathogenesis.

Aerosol challenge

The aerosol challenge technology used among the 3 laboratories was similar. All 3 sites used a head-only Automated Bioaerosol Exposure system contained in a Class 3 Safety cabinet to deliver the target *Y. pestis* aerosol concentration. AGMs were anesthetized and

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the aerosols were generated using a 3-jet Collison nebulizers to attain aerosols of target size of 1 - 3 μ m.

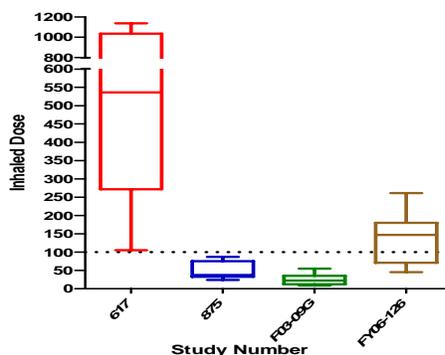
Each animal's estimated inhaled dose was verified retrospectively by collecting atmospheric samples of the aerosol directly from the exposure chamber using an in-line all glass impinger. Impinger samples were enumerated by plating serial dilutions on tryptic soy agar or blood agar plates. Whole body plethysmography was performed in real time (Lovelace, Battelle) or just prior to the aerosol challenges (USAMRIID). Each animal's respiratory parameters were measured which included inhaled minute volume, total accumulated tidal volume (TATV) and aerosol exposure time. The estimated inhaled dose of organisms for each animal was calculated using the formula:

$$\text{Inhaled Dose} = (C \times V)(S \times T)^{-1}(\text{TATV})$$

Where, C is the impinger concentration (CFU/mL), V is the impinger volume (mL), S is the sampling rate (L/min), T is the exposure time (min) and TATV is the total accumulated tidal volume.

The number of LD₅₀ equivalents was calculated by dividing the total inhaled dose for each animal. Each study used a different LD₅₀ number (). For the purposes of this review, the LD₅₀ used was standardized across studies and was based on the study conducted by USAMRIID which determined the LD₅₀ to be [] CFU in AGMs (see Section 4.3.1. above). Overall, there was considerable variability in the targeted aerosol dose exposure for all animals in the 4 studies (Figure 7).

Figure 7: Comparison of the Inhaled dose (LD₅₀ equivalent) across studies



An aerosol challenge dose of 100 \pm 50 LD₅₀ of *Y. pestis* CO92 was targeted in each study. This target aerosol dose appears acceptable based on the median lethal dose study which showed that 99% of the animals that succumbed to the infection had aerosols that were greater than [] CFU for the *Y. pestis* CO92 (equivalent to 40LD₅₀). However, Battelle #617-G607610 had estimated inhaled doses of 105 – 1138 LD₅₀ which were higher than the target challenge of 100 LD₅₀ (Table 18 and Figure 7). It is also important to note that the Battelle #617-G607610 study was a 10- to 100-fold higher than the LD₅₀ equivalents observed for other studies. The calculated LD₅₀ doses in the Battelle #617-G607610 were

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due to high starting nebulizer concentrations and significant variation in the *Y. pestis* aerosol generation. The inhaled dose for the USAMRIID study and Battelle #875-G607610 were the lowest which ranged from 9 – 55 LD₅₀ and 24 – 87 LD₅₀, respectively.

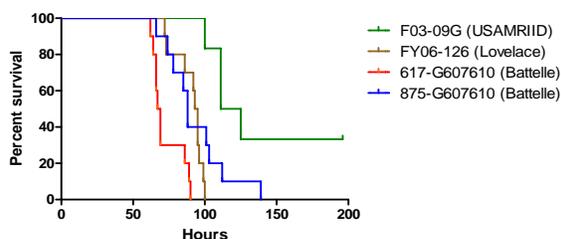
Table 18: Summary of actual exposure doses of *Y. pestis* strain CO92 by study

Parameter	617 (Battelle) n = 10	875 (Battelle) n = 10	F03-09 (USAMRIID) n = 6	FY06-126 (Lovelace) n = 10
Estimated Inhaled Dose in CFU: Mean x 10⁴ ± SD (Range)	20.9 ± 13.1 (3.6 – 39.02)	1.6 ± 7.8 (0.8 – 3.0)	0.86 ± 0.57 (0.3 – 1.9)	4.7 ± 2.4 (1.5 – 8.9)
LD₅₀ Dose: Mean ± SD (Range)	608.5 ± 383 (105 – 1138)	47.6 ± 22.8 (24 – 87)	25.1 ± 16.5 (9 – 55)	137.4 ± 69.7 (45 – 261)
Aerosol Diameter: MMAD ± SD (Range)	2.9 ± 0.07 (2.83 – 3.03)	3.4 ± 0.08 (3.2 – 3.5)	1.2	1.4 ± 0.17 (1.16 – 1.72)
Nebulizer Concentration: Log10 ± SD (Range)	8.32 ± 8.15 (7.38 – 8.66)	7.39	6.48	7.35 ± 7.22 (7.02 – 7.81)
MMAD = median mass aerodynamic diameter; GSD = Geometric Standard Deviation				

Survival

Infectious doses ranging from 7203 to 403,900 CFU of *Y. pestis* bacilli (equivalent to 21 – 1154 LD₅₀) were shown to cause infection and subsequent mortality (Table 19). There were 23 animals found dead and 9 animals that were euthanized for moribund condition. All animals in the Lovelace (as shown in the graph as the brown line) and Battelle studies (shown in the graph as the blue and red lines) succumbed to the plague infection at approximately 62 – 139 hours post-challenge, with challenge doses that ranged from 24 – 1138 LD₅₀ (Figure 8). Two animals (#V605 and #V521) in the USAMRIID study (shown in the graph as the green line) that received lower challenge doses (9 and 13 LD₅₀, respectively) did not become symptomatic and survived. The results suggest that to ensure AGMs achieve a lethal pneumonic plague infection due to *Y. pestis* CO92; individual animal exposures should at least be 40 LD₅₀.

Figure 8: Survival of AGMs after aerosol challenge with *Y. pestis* CO92 strain



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Table 19: Summary of outcome of the 36 AGMs across studies challenged to *Y. pestis* CO92 strain via aerosol route

Study Number	Animal ID	Gender	Inhaled Dose (LD ₅₀ equivalents)*	Time to Death (hours)	Outcome
F03-09G	V113	F	21	125	FD
F03-09G	V514	F	29	111	EU
F03-09G	V521	F	13	--	S
F03-09G	V569	M	23	100	FD
F03-09G	V605	M	9	--	S
F03-09G	V627	M	55	111	EU
FY06-126	X532	M	166	95	EU
FY06-126	X538	M	170	93	EU
FY06-126	X666	F	210	96	EU
FY06-126	X705	F	163	86	FD
FY06-126	X756	M	261	95	EU
FY06-126	X774	F	59	72	EU
FY06-126	X775	M	75	92	EU
FY06-126	X784	M	93	100	EU
FY06-126	X789	F	131	99	FD
FY06-126	X790	F	45	73	FD
617	X106	M	1087	85.8	FD
617	X396	M	1138	61.6	FD
617	X421	F	563	67.4	FD
617	X434	F	829	64.2	FD
617	X511	F	231	65.5	FD
617	X515	F	1018	68.9	FD
617	X606	M	105	89.2	FD
617	X711	F	510	68.8	FD
617	X759	F	285	90.4	FD
617	X770	F	320	66.0	FD
875	W904	F	37	112.3	FD
875	X486	M	42	87.5	FD
875	X603	F	38	88.2	FD
875	X753	F	75	102.6	FD
875	X840	M	24	100.7	FD
875	X900	M	36	139.0	FD
875	X950	M	25	84.7	FD
875	Y212	F	35	74.0	FD
875	Y213	F	75	77.6	FD
875	Y256	M	87	66.3	FD

Abbreviations: F = female; M = male; EU = euthanized due to moribund condition; FD = found dead; S = survived

*The calculations for the LD₅₀ dose in this table was based on an LD₅₀ = [redacted] CFU

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Telemetry: Temperature, Respiratory, Heart and Pulse

Telemetric monitoring devices were implanted in a similar fashion across all studies to remotely capture physiological parameters such as body temperature, heart rate, respiratory rate, cardiovascular function, blood pressure and electrocardiographic signals.

However, measurements for respiratory, heart rates and pulse rates were recorded differently across studies and information for these parameters was not collected in a standard format. For instance, for the Lovelace study, respiratory rates were recorded by intra-thoracic pressure changes analyzed as inspirations and expirations per minute whereas USAMRIID recorded respirations manually per minute. Individual animal data listings in the Battelle studies were provided as breaths per minute. Measurements such as respiratory rates appear to be significant as a late finding. There was no consistent pattern observed for other measurements such pulse and heart rates in each of the studies. The most consistent and apparent telemetry parameter as a marker for disease in AGMs was an elevated temperature across studies.

Each laboratory utilized a different definition of fever onset. For instance, USAMRIID study (F03-09G) defined fever onset as a temperature elevation of $>1.5^{\circ}\text{C}$ for 2 hours above the hourly average baseline. The Lovelace study (FY06-126) defined fever as temperatures $\geq 39^{\circ}\text{C}$ for 2 hours. Onset of fever in the Battelle studies was defined as an hourly temperature of $> 1.5^{\circ}\text{C}$ above the baseline temperature for > 2 hours. Telemetry data for fever was recorded every 30 minutes for USAMRIID and every 5 or 15 minutes for Lovelace and Battelle studies, respectively.

A review of individual animal temperature showed that AGMs exhibited fever onset that ranged from 38 to 84 hours post-challenge (Table 20). Most AGMs had body temperatures that remained elevated until the animals succumbed to the infection. AGMs in the Battelle study# 617-G607610 presented with fever at earlier times compared to other studies. The 2 animals in the USAMRIID study that received $<20 \text{ LD}_{50}$ challenge dose (#V605 and V521) did not show any signs of fever or a temperature change in their daily biorhythm. Overall, a definition of temperatures greater than 39°C for 2 hours correlated with a temperature elevation of $>1.5^{\circ}\text{C}$ for 2 hours above the average baseline. The interval between onset of fever and death varied from 16 to 57 hours.

Bacteremia

Most studies assessed for bacteremia daily, in addition, terminal blood samples were collected from animals that succumbed to the infection. The Battelle studies collected blood for microbiological evaluation in tubes containing sodium polyanethol sulfonate (SPS) as an anticoagulant. In comparison, the Lovelace study collected in tubes containing EDTA as an anticoagulant. It is important to note that the use of EDTA as an anticoagulant is known to decrease bacterial counts which may increase the incidence of false negative results. Blood samples for microbiological evaluation in the Battelle studies were stated to

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be processed within 2 hours of collection; no information was provided for the remaining studies.

All studies performed quantitative evaluation of the blood samples by plating serial dilutions onto tryptic soy agar or blood agar plates. For Battelle studies, qualitative cultures were streaked on Congo Red agar or CIN agar and were visually inspected for colony morphology consistent with *Y. pestis* (i.e. growth and pigmentation). One study (#875-G607610) also used enrichment cultures for qualitative assessments. All plates were incubated at $26 \pm 2^{\circ}\text{C}$ for 36 to 98 hours. There were no differences observed in the culture results based on the method used, the results obtained from quantitative cultures were in agreement with the results of qualitative cultures.

Viable *Y. pestis* colonies were obtained from blood samples collected as early as 18 hours post-challenge (Table 20). The first positive culture in most animals included counts as low as 33 CFU/mL to as high as 657,000 CFU/mL. All AGMs remained positive during the course of the study and at the time of death. Overall, most terminal blood cultures had $>10^4$ CFU/mL of *Y. pestis* isolated. The terminal bacteremia levels were lower in the Lovelace study ($10^2 - 10^6$ CFU/mL) than those seen in other studies ($>10^4$ CFU/mL), though the significance of this observation is unknown.

The 4 studies showed that all AGMs were bacteremic at the time of being febrile. Most animals were bacteremic from 18 to 94 hours post-challenge. The average time to fever onset and bacteremia appears to be similar across studies. Bacteremia preceded the onset of fever in 41% (14/34) of the animals. However, 20% (7/34) of the animals had blood cultures positive at least 9 hours following the onset of fever. All AGMs had terminal blood cultures that were positive for *Y. pestis*. There appears to be no correlation between the time to bacteremia and the actual inhaled dose at the time of challenge. The delay in culture results for some AGMs suggests that bacteremia may not be an optimal trigger for antimicrobial intervention.

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Table 20: Results of the 36 AGMs challenge dose, time to fever, bacteremia, death and outcome for the 4 natural history studies

Study Number	Animal ID	Gender	Challenge Dose (LD ₅₀)*	Time to Fever ** (hours)	1 st Bacteremia** (hours)	Time to Death** (hours)	Terminal Bacteremia (CFU/mL)	Outcome
F03-09G	V113	F	21	76	48	125	1 x 10 ⁸	FD
F03-09G	V514	F	29	78	72	111	3 x 10 ⁶	EU
F03-09G	V521	F	13	--	--	--	--	S
F03-09G	V569	M	23	68	72	100	9 x 10 ⁸	FD
F03-09G	V605	M	9	--	--	--	--	S
F03-09G	V627	M	55	68	72	111	8 x 10 ⁷	EU
FY06-126	X532	M	166	84	69	95	6.6 x 10 ²	EU
FY06-126	X538	M	170	73	69	93	2.1 x 10 ²	EU
FY06-126	X666	F	210	75	71	96	9.3 x 10 ²	EU
FY06-126	X705	F	163	53	45	86	1.4 x 10 ⁵	FD
FY06-126	X756	M	261	67	92	95	1.7 x 10 ²	EU
FY06-126	X774	F	59	52	43	72	7.7 x 10 ⁶	EU
FY06-126	X775	M	75	76	94	92	>2.0 x 10 ⁶	EU
FY06-126	X784	M	93	34	70	100	1.3 x 10 ³	EU
FY06-126	X789	F	131	75		99	>2.0 x 10 ⁵	FD
FY06-126	X790	F	45	38	46	73	>2.0 x 10 ⁶	FD
617	X106	M	1087	40	46	85.8	7.7 x 10 ⁶	FD
617	X396	M	1138	44	46	61.6	6.57 x 10 ⁵	FD
617	X421	F	563	47	45	67.4	3.76 x 10 ³	FD
617	X434	F	829	48	45	64.2	8.47 x 10 ³	FD
617	X511	F	231	50	45	65.5	3.97 x 10 ⁴	FD
617	X515	F	1018	44	46	68.9	5.97 x 10 ⁴	FD
617	X606	M	105	43	44	89.2	2.54 x 10 ⁴	FD
617	X711	F	510	44	46	68.8	4.37 x 10 ⁴	FD
617	X759	F	285	43	44	90.4	2.14 x 10 ⁷	FD
617	X770	F	320	43	44	66.0	6.23 x 10 ⁴	FD
875	W904	F	37	56	67	112.3	3.43 x 10 ⁵	FD
875	X486	M	42	59	44	87.5	8.03 x 10 ⁷	FD
875	X603	F	38	52	42	88.2	2.16 x 10 ⁹	FD
875	X753	F	75	63	68	102.6	7.43 x 10 ⁶	FD
875	X840	M	24	46	18	100.7	7.23 x 10 ⁷	FD
875	X900	M	36	62	68	139.0	8.63 x 10 ⁶	FD
875	X950	M	25	48	43	84.7	1.16 x 10 ⁹	FD
875	Y212	F	35	57	67	74.0	4.73 x 10 ⁷	FD
875	Y213	F	75	58	67	77.6	2.41 x 10 ⁸	FD
875	Y256	M	87	48	43	66.3	1.03 x 10 ⁹	FD

*The calculations for the LD₅₀ dose in this table was based on an LD₅₀ = [redacted] CFU

**Based on the information provided in the applicant datasets.

Clinical Observations

Cage side observations which included activity, posture, nasal discharge, respiratory characteristics, inappetence/anorexia, stool characteristics or other abnormalities were reported twice daily post-challenge across all studies. The most commonly observed abnormal behavior was decrease in appetite that started from day 0 to day 3 post-challenge

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depending on the animal. At later stages of the disease, animals were noted to be in the hunched position or had blood discharges from the nose or mouth. However, across the studies, there was no consistent pattern in the onset of abnormal behaviors observed in relation to the progression of disease.

Two studies (Lovelace FY06-126 and USAMRIID F03-09G) performed radiographs of the animals prior to *Y. pestis* challenge, at the onset an increased respiration rate and/or before euthanasia/death. None of the animals in the Battelle studies had thoracic radiographs performed prior to or during the course of infection. For animals that had chest radiographs performed prior to the start of study, animals showed clear radiographic images with the exception of one animal that had a mid-right caudal lobe opacity on film. During the course of infection, 60% of the animals showed radiographic infiltrate which were mostly localized to a single lobe. Radiographs at the time of euthanasia or death showed small infiltrates in multiple lobes, suggesting that the localized lobe had rapidly spread to other lobes consistent of a pneumonitis.

The USAMRIID study (F03-09G) was the only study that assigned a clinical score based on activity, behavior, stimuli response and breathing. These clinical assessments were used mainly in the decision to euthanize animals. The euthanasia criteria for USAMRIID was based on a cumulative score of ≤ 5 including loss of consciousness (comatose), convulsions and abnormal respiration present and/or fluid found radiographically in the lungs. Three animals in the USAMRIID study were euthanized based on this criteria (V627, V514 and V569) and one animal was found dead (V113).

Body weights of AGMs were measured in Battelle study #617-G607610 on day 3 prior to challenge and at the time of necropsy. The results showed that individual AGM body weights did not change significantly post-challenge. No other studies measured body weights.

Animals in the Battelle studies (#617-G607610 and #875-G607610) had serum samples collected in all animals prior to challenge and at the time of death. However, the results of these studies were not provided. No other studies measured serum antibody titers.

Pathology

A complete gross necropsy was performed on all animals in the Lovelace and Battelle studies. In the USAMRID study gross necropsy was performed only on those animals that died or were euthanized for moribund condition. Gross necropsies included physical examination of external surfaces, body cavities and major organ systems. Select tissue samples were collected from the liver, spleen, lungs, intrathoracic lymph nodes (bronchial and mediastinal); some studies had additional samples taken from the brain, heart and kidneys. In addition, any gross lesions observed had also samples collected. All samples were preserved in 10% neutral buffered formalin for histopathologic evaluation. A board certified veterinary pathologist performed histopathologic evaluation. Fixed sections of

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tissues (5 micron thick) were processed to slides and stained with hematoxylin and eosin (H&E). Microscopic findings were graded semi-quantitatively according to the following scale, with the associated numerical score used to calculate average severity grades for each lesion by sex:

- Within normal limits – Tissue considered to be normal, under the conditions of the study considering the age, sex and animal species. Alterations may be present which, under other circumstances, might be considered deviations from normal.
- Grade 1 – minimal; represented the least detectible lesion; The amount of change present barely exceeds that which is considered to be within normal limits
- Grade 2 – mild; represented an easily discernible lesion but of limited severity. The lesion probably does not produce any functional impairment.
- Grade 3 – moderate; the lesion is prominent but there is significant potential for increased severity. Limited tissue or organ dysfunction is possible.
- Grade 4 – marked; the degree of change is either as complete as possible or great enough in intensity or extent to expect significant tissue or organ dysfunction.

Gross pathology findings showed that the most common observed lesions related to infection with *Y. pestis* included the presence of thoracic transudate usually a clear fluid, discoloration of the lymph nodes and lungs. In addition, the pathologist noted the presence of lung masses or lesions in most monkeys.

Microscopically, these masses or lesions represented areas of hemorrhage and/or inflammation in the lungs (Table 21). Bacteria, both within macrophages and extracellularly, were commonly observed in the lungs and associated with suppurative inflammation with variable amounts of fibrin accumulation and occasional edema. In almost all of the lymph nodes examined, especially bronchial lymph nodes, contained bacteria usually associated with hemorrhage and suppurative inflammation. Bacteria were present in most spleens and were usually intracellular. Lymphoid necrosis was occasionally noted in the spleen and/or lymph nodes but was never of greater than mild severity hence was considered most likely to be related to corticosteroid release from the acute stress of illness. One animal in the Battelle 875 showed macroscopic findings in the brain confirmed microscopically to contain bacteria. For Battelle 617, there were no findings in the brain following microscopic examination of any animal. Animal #X106 was noted to have fibrin thrombi in renal glomerular capillaries, consistent with disseminated intravascular coagulopathy. Untreated AGMs had signs of hemorrhagic pneumonia and overwhelming presence of bacteria in the order of $>10^6$ in tissues.

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Table 21: Independent Pathology Report

Gross Observation	USAMRIID F03-09G (n = 4)	Lovelace FY06-126 (n = 10)	Battelle 617-G607610 (n = 10)	Battelle 875-G6076010 (n = 10)	Total (n = 34)
Lung					
Bacteria	4	10	10	10	34
Edema	4	9	10	10	33
Hemorrhage	3	6	8	10	29
Inflammation	4	10	10	6	31
Necrosis	0	0	--	4	4
Pleura, fibrin	3	10	3	8	24
Pleura, inflammatory	0	0	1	0	1
Within Normal Limits	0	0	0	0	0
Lymph Node – bronchial					
Bacteria	4	8	10	10	32
Edema	4	5	3	7	19
Hemorrhage	0	3	8	6	17
Inflammation	3	4	8	4	19
Lymphoid Depletion	0	2	1	10	13
Lymphoid hyperplasia	1	2	0	0	3
Within Normal Limits	0	0	0	0	0
Lymph Node – mandibular ** (only examined 3 animals in 617 and 875)					
Bacteria	0	--	3	1	4
Hemorrhage	0	--	2	1	3
Inflammation	0	--	1	0	1
Lymphoid Depletion	0	--	0	1	1
Lymphoid Hyperplasia	3	--	0	0	3
Within Normal Limits	1	--	0	0	1
Lymph Node – mediastinal					
Bacteria	4	--	10	9	23
Edema	4	--	0	1	5
Hemorrhage	1	--	5	5	11
Inflammation	4	--	7	1	12
Lymphoid Depletion	0	--	0	8	8
Lymphoid Hyperplasia	1	--	0	0	1
Within Normal Limits	0	--	0	1	1
Spleen ** (only 2 examined for 617)					
Bacteria	3	5	9	1	18
Congestion	1	0	0	0	1
Hemorrhage	0	5	4	2	11
Inflammation	4	7	1	0	12
Lymphoid Depletion	0	0	4	2	6
Within Normal Limits	0	1	0	0	1
Liver					
Bacteria	0	1	0	--	1
Congestion	0	1	0	--	1
Hepatocyte, degeneration/hydropic change	1	7	0	--	8
Inflammation	0	1	0	--	1

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Gross Observation	USAMRIID F03-09G (n = 4)	Lovelace FY06-126 (n = 10)	Battelle 617-G607610 (n = 10)	Battelle 875-G6076010 (n = 10)	Total (n = 34)
Sinusoid, thrombi	1	0	0	--	1
Within Normal Limits	1	2	10	--	13
Brain					
Bacteria	--	--	--	1	1
Hemorrhage	--	--	--	1	1
Fibrin Exudation	--	--	--	1	1
Within Normal Limits	4	10	10	9	33
Kidney					
Glomerulus, fibrin	--	--	1		1
Microgranuloma	--	--	1		1
Tubule dilation	1	--	--		1
Tubule dilation	1	--	--		1
Tubule –interstitial, inflammation, chronic	0	--	1	--	1
Within Normal Limits	2	--	8	--	10
Heart					
Myocardium, fibrosis	1	--	1	--	1
Myocardium, cellular infiltrate, lymphoplasmacytic	1	--	--	--	1
Protozoal cyst	--	--	1	--	1
Within Normal Limits	2	--	8	--	10
Thymus					
Bacteria	--	--	--	2	2
Edema	0	--	--	2	2
Hemorrhage	0	--	--	2	2
Lymphoid Depletion	0	--	--	1	1
Within Normal Limits	3	--	--	0	3

Microbiological cultures of tissues were performed in the Battelle study #875-G607610 and Lovelace FY06-126 study. Positive cultures for *Y. pestis* in the lung and nasal fluids were reported in Battelle study #875-G607610. The Lovelace study performed quantitative cultures of the spleen, liver, tracheobronchial lymph node and from lesion and non-lesion areas of the lungs (Table 22). Culture results showed that the tissue burden of *Y. pestis* was high particularly in the lung especially taken from lesion areas (range, 10^7 - 10^9 CFU/g of tissue), however, bacterial load in the range of 10^5 to 10^8 CFU/g of tissue were also reported from the tracheobronchial lymph nodes, liver and spleen.

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Table 22: Bacterial load observed in tissues from monkeys infected with *Y. pestis* CO92 strain in the Lovelace study (FY06-126)

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

4.3.3. Efficacy Study

This was a single pivotal efficacy study performed in AGMs that evaluated the efficacy of levofloxacin under the Animal Rule (21 CFR 314.610) to support a treatment indication for inhalational plague in adults and pediatric patients (>50 kg and \geq 6 months of age). The study was conducted at Lovelace Respiratory Research Institute (Albuquerque, NM) in compliance with U.S. FDA Good Laboratory Practice Regulations.

Animals

Adult AGMs of unknown age weighing 3.66 to 6.81 kg were wild caught from a colony on the island of St. Kitts Island, West Indies (Alpha Genesis, Yemassee SC). Animals were given a physical examination at quarantine and when transferred to the Lovelace Respiratory Research Institute (LRRRI). Animals were pre-screened for B-virus, Simian Immunodeficiency virus (SIV), Simian Retrovirus (SRV), Simian T-cell Leukemia virus (STLV), hepatitis B virus (HBV), and tuberculosis. Fecal screens were performed periodically on all animals; however, it is unknown whether the screens included prior exposure to *Yersinia* organisms. Monkeys were treated with ivermectin and administered antibiotics and vaccinations prior to placement on the study protocol. All animals that were included in the “in-life” phase study protocol did not receive systemic antibiotics within 28 days prior to challenge or topical mupirocin ointment within 14 days of aerosol exposure. A physical examination and complete blood counts (CBC), full chemistry panel and baseline chest radiographs were performed and reviewed by a veterinarian to ensure that the animals were healthy prior to assigning animals to the study. Only experimentally naïve AGMs that had passed a physical examination within 30 days were assigned to the protocol.

Prior to in-life phase study, all animals were surgically implanted with telemetry monitoring devices (T30F telemeters, DISS Inc.) for continuous monitoring of body temperature, intra-thoracic pressures, respiratory rates, heart rates and basic electrocardiographic signals. Baseline data were collected for 7 days prior to challenge. All

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animals were also implanted with central venous catheters inserted into the right femoral vein which exited through the skin of the upper back of the monkey. For cohort 1 and 2 single lumen Broviac catheters were used and for cohort 3 Hickman Dual Ports catheters were used. All animals wore non-restraining jackets to limit animal's access to the catheter or exit site. The monkeys were maintained in individual stainless steel cages in a non-human primate isolation facility and were provided water *ad libitum*; fresh feed was provided twice daily.

Challenge Organism

Y. pestis CO92 provided by (b) (4) the University of New Mexico was used in the experimental efficacy study. The *Y. pestis* CO92 used in the study was similar to the challenge organism used in the natural history studies. Characterization studies and checks for purity of the seed and working stocks used were based on Gram stain, colony morphology and phenotype on different media. In addition, stock cultures were confirmed to be pathogenic in mice prior to the preparation of the aerosol suspension. The working stock culture was prepared on March 2, 2008 of a seed stock prepared on October 28, 2005. The LD₅₀ of aerosolized *Y. pestis* CO92 in AGMs has been calculated to be [redacted] CFU (see Section 4.3.1.). The levofloxacin MICs against the CO92 strain was determined to be 0.03 µg/mL.

Aerosol Preparation and Challenge

The aerosol suspension for challenge was prepared from a fresh culture of *Y. pestis* CO92 obtained from the working stock cultures.

Similar to the aerosol procedures used in the natural history study, anesthetized AGMs were challenged via a head only Automated Bioaerosol Exposure system contained in a Class 3 Biosafety cabinet. A 3-jet Collison nebulizer was used to generate a controlled delivery of aerosols from a liquid suspension of *Y. pestis* with a target particle size of 1 – 3 µm in diameter. Animals estimated inhaled dose was verified retrospectively by collecting aerosol samples using an in-line all glass impinger. Whole body plethysmograph was performed in real time during the aerosol challenge to determine each animal inhaled minute volume and total accumulated tidal volume. The duration of each aerosol exposure was directly related to animal minute volume to achieve the target challenge dose. The estimated dose was calculated using the formula as described for the natural history studies (see Section 4.3.2).

Experimental Design

The study was investigator-blinded, randomized, placebo-controlled, performed prospectively at a single center. Investigator-blinded meant that the investigator making decisions for euthanasia was blinded to the treatment category. Animals were randomized as to exposure to infectious aerosols based on aerosol challenge day, order of aerosol challenge and treatment group. Each cohort was challenged via a head-only aerosol inhalation to achieve a *Y. pestis* CO92 target dose of 100 ± 50 LD₅₀. The challenge was

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performed in 3 iterations on March 25, 2008 (n= 8), May 2, 2008 (n = 8) and January 23, 2009 (n= 10). Temperatures were taken every 30 minutes throughout the study via telemetry (temperature datasets were provided). Treatment was initiated when the animal body temperature was $>39^{\circ}\text{C}$ for at least one hour. Levofloxacin-treated animals were administered 8 mg/kg (high dose) levofloxacin followed by a second infusion within 12 hours of 2 mg/kg levofloxacin (low dose). Control animals were administered similar volumes of 5% dextrose. The treatment regimen was given over a maximum of 10 days for a total of 20 doses.

The primary efficacy outcome was survival at study termination on day 28. The intention-to-treat population was used as the primary analyses population. AGMs were evaluated and monitored for clinical signs at various time intervals and changes in temperature via continuous telemetry averaged hourly during the study. Cage side observations (food consumption, behavior, activity) were documented twice daily. For cohorts 1 and 2, radiographs were performed at baseline, Day 5/6 post-challenge and prior to euthanasia or at the end of study on Day 28. Cohort 3 animals had radiographs performed only at baseline. Blood for hematology, clinical chemistry and pharmacological (serum peak/trough) assessments were obtained at various intervals.

Whole blood for microbiological evaluation was collected via the catheter or femoral vein and collected in 1.5 mL isolator tubes containing EDTA. Blood was taken at the time of challenge, for the first 6 days post-challenge or until clearance of *Y. pestis* organisms were confirmed and weekly thereafter. Terminal blood cultures were collected in monkeys that became a candidate for euthanasia or survived to study termination on Day 28 post-challenge. Cohort 3 animals had an additional blood specimens obtained prior to infusion, on day 28 blood samples were collected from the femoral vein and catheter as well as the catheter tip was cultured. For quantitative evaluation, blood was serially cultured onto tryptic soy agar plates and incubated at 28°C for 48 to 72 hours (100 μL /plate, 3 plates/sample). For qualitative evaluation, undiluted samples were streaked for isolation on Congo Red agar plates and visually inspected for colony morphology consistent with *Y. pestis*.

Gross necropsies were performed on all animals that were found dead or euthanized during the study including survivors on day 28 post-challenge. Tissues for histopathology examination were processed and fixed similar to the procedures described in the natural history studies. All necropsies and histopathologic evaluations were performed by a board certified pathologist. An independent pathology working group at NIH also reviewed the histological slides. Both pathology groups graded the severity of microscopic findings using a semi-quantitative scale as described in the natural history studies. Microbiological evaluation was performed on tissues samples obtained from the spleen, liver, tracheobronchial lymph node and from lesion and non-lesion areas of the lungs.

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RESULTS

Prior to randomization, one animal (X717) was removed from the study due to health problems. During post-randomization, another animal (X779) was removed from the study due to protocol violation because the animal received treatment prior to development of a fever. The efficacy study was conducted initially in 2 iterations with the first cohort challenged on March 25, 2008 and the second on May 2, 2008 (Table 23). The applicant stated that it was not possible to document the circulating plasma levofloxacin concentration since a single lumen catheter was used (Broviac) for dosing and obtaining blood samples for cohorts 1 and 2. A third cohort was added to the protocol on January 23, 2009 to address this problem in which animals had a dual-lumen catheter (Hickmann) inserted for collection of blood and plasma samples. The results of the 24 AGMs were provided in which 7 animals were administered placebo and 17 animals levofloxacin treatment.

Table 23: Levofloxacin Efficacy Study Design

Groups	Cohort 1 (March 25, 2008)	Cohort 2 (May 2, 2008)	Cohort 3 (January 23, 2009)	Total
<i>Y. pestis</i> aerosol challenge	8	7	10	25
Total Randomized	8	7*	10	24
Placebo (Controls)	3	2	2	24
Levofloxacin Treatment	5	4	8	

*Animal #779 removed from the study after randomization

Aerosol Challenge

The applicant stated that pre-study of bio-aerosol runs determined that in order to attain the desired challenge dose of 100 ± 50 LD₅₀, a starting titer of 2.7×10^7 CFU/mL (7.3 ± 0.5 log₁₀ CFU/ml) would have to be achieved in the study. The time of exposure and spray factor for each animal was adjusted such that a target inhaled dose of 100 ± 50 LD₅₀ was based on an expected *Y. pestis* aerosol concentration generated in approximately 5.6 L of air. Thus, in order to achieve a target aerosol challenge dose of 100 LD₅₀ [REDACTED] CFU) in 5.6 L of air, it is estimated that the aerosolized concentration of *Y. pestis* would range from [REDACTED] (i.e., [REDACTED] CFU/5.6L of air).

Based on individual animal data, all animals were exposed to infectious aerosols within the specified target range (1 – 3 μm) with an overall median mass aerosol diameter (MMAD) of 2.23 ± 0.13 μm that ranged from 1.92 to 2.41 μm, suggesting that there was deposition of the aerosol in the lower respiratory tract (Table 24). The concentration of *Y. pestis* in the nebulizer suspensions determined prior to or after aerosol generation from cohorts 1 and 2 were within the acceptable limits of 7.3 ± 0.5 log₁₀ CFU/ml whereas 6 animals from cohort 3 (Y160, Y295, Y275, Y276, Y226 and Y301) had below the acceptable limit. Most of the animals had aerosols concentrations within the specified target range of 3,125 to 9,321 CFU/L. Differences were stated to be due to a technical dilution error which may have occurred when the actual aerosol samples were enumerated for these animals at the time of challenge.

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Table 24: Summary of nebulizer and estimated aerosol concentration at the time of challenge in 24 AGMs

Cohort	Animal ID	Gender	MMAD	Actual Nebulizer concentration (CFU/mL) (Pre)	Actual Nebulizer concentration (CFU/mL) (Post)	Actual Spray Factor (Post)	Estimated Aerosolized Concentration
1	X702	F	2.14	1.72 x 10 ⁷	1.90 x 10 ⁷	2.07 x 10 ⁻⁷	3933
1	X773	M	2.20	1.64 x 10 ⁷	2.08 x 10 ⁷	2.73 x 10 ⁻⁷	5678
1	X762	M	2.19	1.49 x 10 ⁷	1.90 x 10 ⁷	2.96 x 10 ⁻⁷	5624
1	X663	F	2.20	1.52 x 10 ⁷	2.08 x 10 ⁷	2.53 x 10 ⁻⁷	5262
1	X662	F	2.21	1.47 x 10 ⁷	1.90 x 10 ⁷	3.31 x 10 ⁻⁶	62890
1	X648	F	2.15	1.27 x 10 ⁷	2.16 x 10 ⁷	2.01 x 10 ⁻⁷	4342
1	X437	M	2.27	1.44 x 10 ⁷	1.69 x 10 ⁷	1.54 x 10 ⁻⁷	2603
1	X523	M	2.24	1.48 x 10 ⁷	1.81 x 10 ⁷	3.37 x 10 ⁻⁷	6100
2	U193	F	2.05	1.67 x 10 ⁷	1.78 x 10 ⁷	4.97 x 10 ⁻⁷	8847
2	X734	M	2.09	1.92 x 10 ⁷	1.90 x 10 ⁷	6.68 x 10 ⁻⁷	12692
2	X732	F	2.23	2.06 x 10 ⁷	1.97 x 10 ⁷	4.84 x 10 ⁻⁷	9535
2	X419	F	2.14	1.84 x 10 ⁷	1.83 x 10 ⁷	5.01 x 10 ⁻⁷	9168
2	X771	M	2.07	2.05 x 10 ⁷	1.78 x 10 ⁷	5.06 x 10 ⁻⁷	9007
2	X761	M	2.14	1.81 x 10 ⁷	1.90 x 10 ⁷	4.36 x 10 ⁻⁷	8284
2	X779*	F	2.07	--	6.35 x 10 ⁶	3.43 x 10 ⁻⁷	6675
3	X888	F	2.36	7.67 x 10 ⁶	9.63 x 10 ⁶	3.60 x 10 ⁻⁷	3467
3	Y283	M	2.37	6.70 x 10 ⁶	8.50 x 10 ⁶	3.78 x 10 ⁻⁷	3213
3	Y160	F	2.38	3.43 x 10 ⁶	5.00 x 10 ⁶	8.78 x 10 ⁻⁸	439
3	Y217	F	2.36	7.87 x 10 ⁶	7.87 x 10 ⁶	3.27 x 10 ⁻⁷	2573
3	Y226	F	2.36	5.00 x 10 ⁶	6.90 x 10 ⁶	1.12 x 10 ⁻⁷	773
3	Y295	F	2.41	8.70 x 10 ⁶	7.33 x 10 ⁵	2.97 x 10 ⁻⁷	218
3	Y275	M	2.40	5.47 x 10 ⁶	5.33 x 10 ⁶	4.24 x 10 ⁻⁸	226
3	Y276	M	1.92	5.07 x 10 ⁶	2.57 x 10 ⁶	6.52 x 10 ⁻⁸	168
3	Y293	M	2.34	6.80 x 10 ⁶	1.04 x 10 ⁷	4.20 x 10 ⁻⁷	4368
3	Y301	M	2.35	6.20 x 10 ⁶	6.03 x 10 ⁶	3.35 x 10 ⁻⁸	202

MMAD = mass median aerosol diameter

*Excluded from the study post-randomization

The corresponding LD₅₀ equivalent in all animals challenged ranged from 3 to 148LD₅₀ (Table 26). Overall, there was no difference in challenge dose among the treatment groups with regards to aerosol exposure (Figure 9A). However, the average LD₅₀ dose varied across the days challenged with an average dose of 74.2 ± 31.03 LD₅₀, 124.4 ± 10.5 LD₅₀ and 22.1 ± 23.1 LD₅₀, for cohorts 1, 2 and 3 respectively (Table 25). Animals in cohort 3 received significantly fewer organisms than animals in cohorts 1 or 2 (Figure 9B). Six levofloxacin treated animals (#Y160, Y226, Y295, Y275, Y276 and Y301) from cohort 3 had aerosol exposures that ranged from 3 to 12 LD₅₀.

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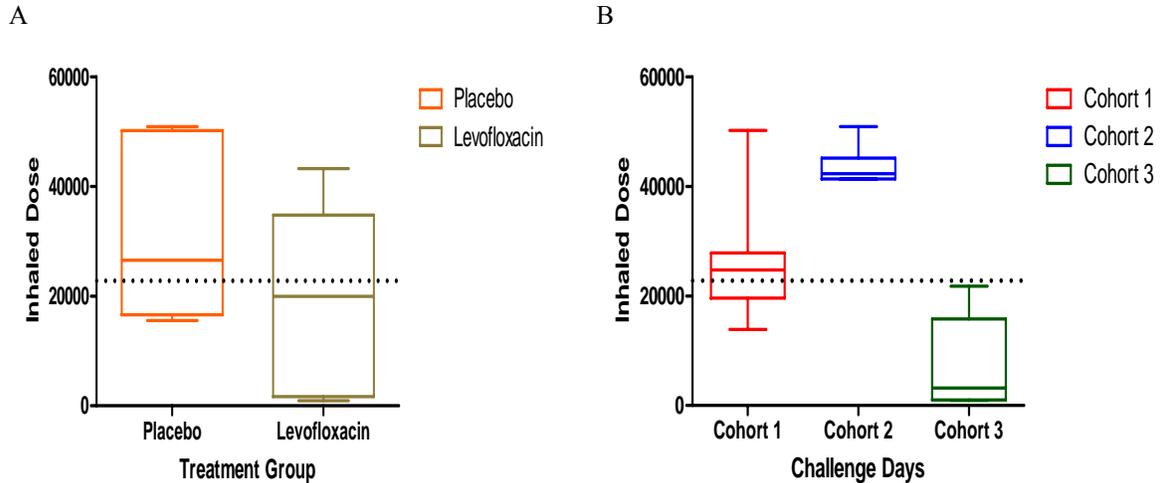
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Table 25: Summary of the aerosol challenge dose in the 24 AGMs using the *Y. pestis* strain CO92

Parameter	Treatment Groups		Cohorts*		
	Placebo (n = 7)	Levofloxacin (n = 17)	Cohort 1 (n = 8)	Cohort 2 (n = 6)	Cohort 3 (n = 10)
Estimated Inhaled Dose: Mean CFU x 10⁴ ± SD (Range)	3.17 ± 1.58 (1.6 – 5.1)	1.91 ± 1.61 (0.09 – 4.3)	2.60 ± 1.09 (1.4 – 5.0)	4.35 ± 0.37 (4.1 – 5.1)	0.78 ± 0.81 (0.09 – 2.2)
LD₅₀ Dose: Mean ± SD (Range)	90.4 ± 45.04 (44– 145)	54.6 ± 45.9 (3 – 123)	74.2 ± 31.03 (40 – 143)	124.4 ± 10.5 (118 – 145)	22.1 ± 23.1 (3 – 62)
Aerosol Diameter: MMAD ± GSD (Range)	2.20 ± 0.05 (2.05 – 2.37)	2.24 ± 0.03 (1.92 – 2.41)	2.20 ± 0.04 (2.14 – 2.27)	2.12 ± 0.06 (2.05 – 2.23)	2.32 ± 0.14 (1.92 – 2.41)
Nebulizer Concentration: Log₁₀ ± SD (Range)	7.19 ± 0.16 (6.92 – 7.32)	6.98 ± 0.40 (5.86 – 7.34)	7.29 ± 0.04 (7.22 – 7.34)	7.27 ± 0.02 (7.25 – 7.30)	6.71 ± 0.35 (5.86 – 7.02)

MMAD = median mass aerodynamic diameter; GSD = Geometric Standard Deviation
*Cohort 1 = March 25, 2008; Cohort 2 = May 2, 2008; Cohort 3 = January 23, 2009

Figure 9: Mean inhaled dose by challenge day and treatment groups



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Table 26: Actual Exposure doses of *Y. pestis* CO92 strain by animal

Cohort	Animal ID#	Gender	Impinger Concentration (CFU/mL) [C]	Impinger sample (mL) [V]	CFU Recovered (CFU) [C]*[V]	Sampling rate (L/min) [S]	Exposure Time (min) [T]	Sample volume (L) [S*T]	Calculated CFU per inhaled volume (CFU/L) [(CV)/(ST)]	Total Accumulated Tidal volume (L) [TATV]	Estimated Inhaled Dose (CFU) [(CV)/(ST)]* TATV	Calculated LD ₅₀ equivalents [Inhaled dose/
1	X702	F	9530	18.89	179450	5.101	8.983	46	3928	4.97	19523	57
1	X773	M	14500	18.69	271150	5.080	9.370	48	5693	8.811	50164	146
1	X762	M	12600	18.65	235872	5.099	8.183	42	5632	4.703	26488	77
1	X663	F	13500	18.81	252990	5.120	9.383	48	5287	4.394	23232	68
1	X662	F	22600	18.67	422620	5.145	13.000	67	6309	4.475	28234	82
1	X648	F	12000	18.83	225720	5.167	10.050	52	4352	4.568	19881	58
1	X437	M	5770	18.54	106803	5.102	8.017	41	2616	5.307	13883	41
1	X523	M	18000	18.72	336960	5.102	10.817	55	6106	4.305	26288	77
2	U193	F	23300	18.76	436642	4.922	10.017	49	8863	4.793	42482	124
2	X734	M	54700	18.12	991711	4.902	15.900	78	12715	3.999	50846	148
2	X732	F	29000	18.24	529250	4.900	11.300	55	9554	4.524	43222	126
2	X419	F	25300	18.30	463243	4.943	10.233	51	9154	4.592	42033	123
2	X771	M	21400	18.55	397612	4.559	9.683	44	8993	4.591	41287	120
2	X761	M	15800	18.92	299568	4.834	7.450	36	8302	4.977	41321	120
2	X779	F	21300	18.35	390855	5.985**	11.967	191.29	6675	4.352	29050	85
3	X888	F	10800	18.43	198612	5.246	10.917	57	3475	4.472	15539	45
3	Y283	M	5130	18.42	94443	5.227	5.620	29	3217	5.16	16599	48
3	Y160	F	867	18.45	16022	5.315	6.867	36	438	4.819	2113	6
3	Y217	F	4600	18.61	85560	5.288	6.283	33	2576	5.124	13202	38
3	Y226	F	1500	18.60	27930	5.151	7.000	36	774	5.437	4207	12
3	Y295	F	467	18.16	8485	5.235	7.433	39	218	4.773	1040	3
3	Y275	M	400	18.80	7520	5.340	6.233	33	226	5.536	1251	4
3	Y276	M	233	18.50	4306	5.243	4.917	26	167	5.504	920	3
3	Y293	M	8830	18.35	161854	5.333	6.933	37	4381	4.968	21767	64
3	Y301	M	367	18.47	6790	5.143	6.517	34	202	4.573	925	3

Note: The calculations for the LD₅₀ dose in this table was based on an LD₅₀ [redacted] CFU. The applicant calculation was based on an LD₅₀ that is equal [redacted] CFU;

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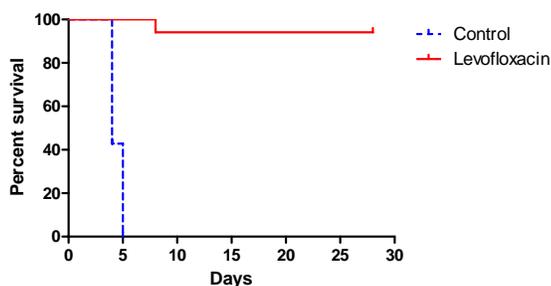
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Survival

All of the control animals succumbed to the infection within 105.3 ± 14.5 hours post-challenge (Figure 10). Four controls were found dead (#X702, X734, X888 and Y283) and 3 animals (X773, X762 and U193) were euthanized due to moribund condition. In the levofloxacin-treated group, 17 of 18 (94%) animals survived to day 28. One animal (#Y160) was euthanized due to moribund condition on day 9 post-challenge for gastric complications unrelated to plague (based on the study pathologist report).

Figure 10: Survival of 24 AGMs after aerosol challenge with *Y. pestis* CO92 strain



Telemetry: Temperature

The diurnal temperature patterns were recorded in all animals at baseline and post-challenge. For cohort 2, telemetry was not continuously monitored between the 11th and 12th day. The applicant stated that approximately a 15-hour lapse occurred on these days due to the time exceeded the 99 hour time limit prior to being reset. These errors occurred late in the experiment and not deemed of sufficient severity to affect the analyses.

The trigger for treatment was onset of fever defined as an increase in body temperature $\geq 39^{\circ}\text{C}$ for at least 1 hour post-challenge. The time interval from *Y. pestis* aerosol exposure to fever onset ranged from 52 to 165 hours post challenge (Table 27). Most animals developed a fever on average at 77 hours (day 3). Two animals developed fever within 96 hours or on day 4 post-challenge (#X702 and Y275), 2 animals on day 5 (#X437 and Y295) and one animal on day 7 (#Y276). Control animals had an increase in temperature that ranged from 58 to 93 hours post-challenge, similar to that observed in natural history studies (39 – 90.5 hours). The variations in LD₅₀ dose (range, 3 to 145 LD₅₀) did not appear to alter the infection and disease progression in the monkeys. Overall, there was no association between the actual challenge dose and time to fever onset across the cohorts (Figure 11).

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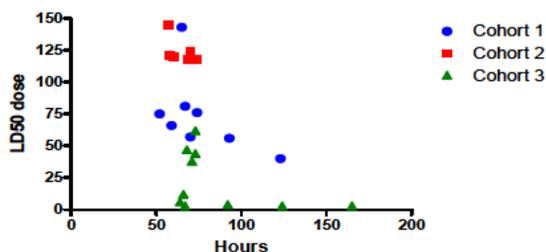
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Figure 11: Comparison of Challenge dose to fever onset



Across Cohorts, $p < 0.12$ Chi-Square

Table 27: Fever onset and resolution in AGMs exposed to *Y. pestis* CO92 strain

Animal ID	Cohort	Treatment	LD ₅₀ equivalents	Fever Onset* (hours)	Time to Treatment (hours)	Fever Resolution** (hours)
X702	1	Control	56	93	94	--
X773	1	Control	143	65	69	--
X762	1	Control	76	74	75	--
X663	1	Levofloxacin	66	59	60	18
X662	1	Levofloxacin	81	67	68	13
X648	1	Levofloxacin	57	70	72	30
X437	1	Levofloxacin	40	123	125	41
X523	1	Levofloxacin	75	52	57	47
U193	2	Control	121	58	60	--
X734	2	Control	145	57	61	--
X732	2	Levofloxacin	124	70	72	34
X419	2	Levofloxacin	120	60	66	58
X771	2	Levofloxacin	118	74	75	6
X761	2	Levofloxacin	118	69	71	27
X888	3	Control	44	73	77	--
Y283	3	Control	47	68	73	--
Y160	3	Levofloxacin	6	64	70	48
Y217	3	Levofloxacin	38	71	76	103
Y226	3	Levofloxacin	12	66	70	9
Y295	3	Levofloxacin	3	124	129	21
Y275	3	Levofloxacin	4	92	97	53
Y276	3	Levofloxacin	3	165	170	70
Y293	3	Levofloxacin	62	73	77	(3)
Y301	3	Levofloxacin	3	67	72	26

*Fever defined as $\geq 39^{\circ}\text{C}$ for more than 1 hour as recorded by telemetry.

**Fever Resolution determined from the time of treatment at which the animal had a last recorded temperature of 39°C

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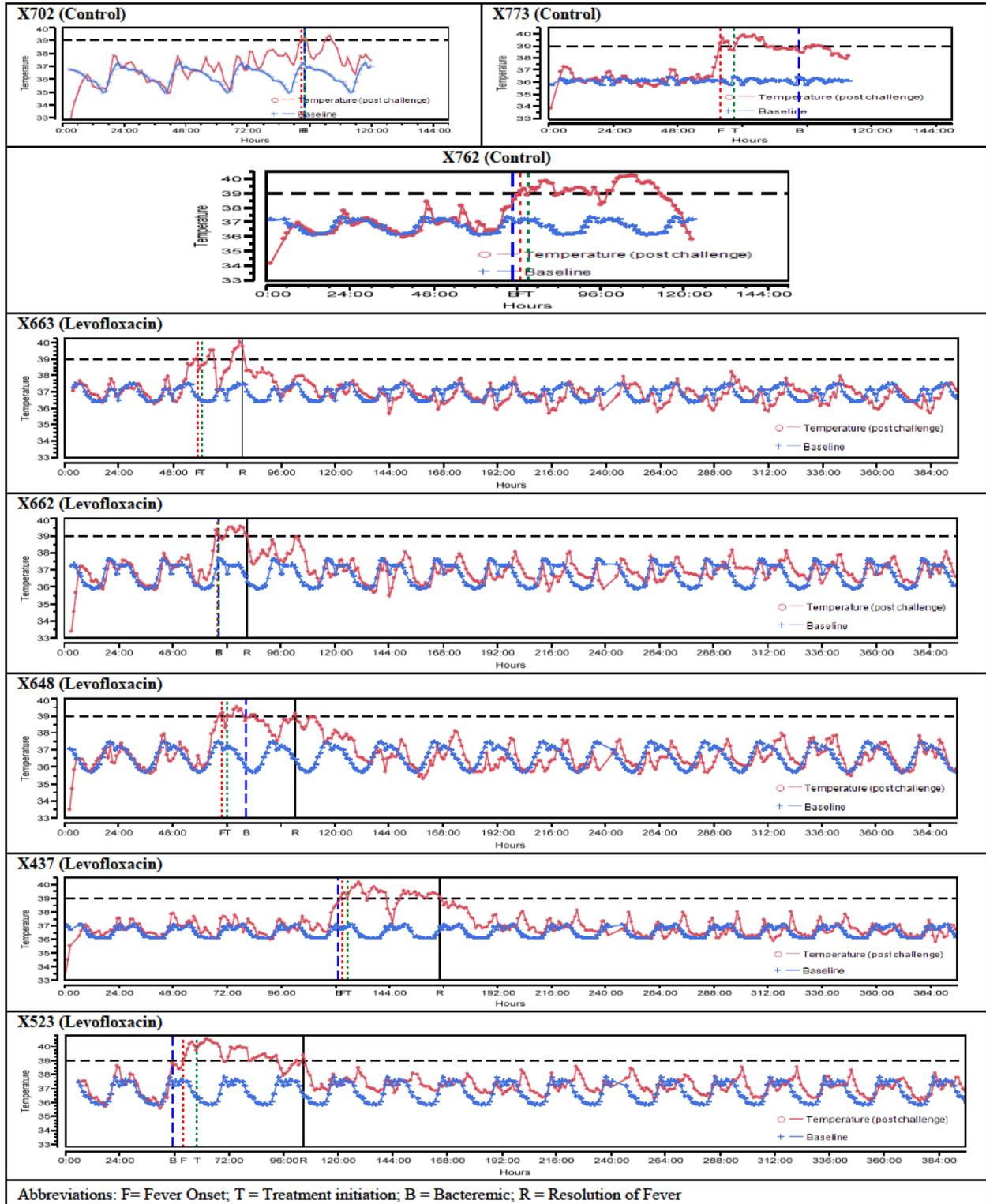
In order to be consistent with the natural history studies by accounting for diurnal variations in temperature in each animal, each animal average hourly baseline temperature was assessed. A review of the animal data was performed to determine whether the onset of fever defined as a consistent increase in temperature of $\geq 1.5^{\circ}\text{C}$ above the baseline for 2 hours was achieved in the animals of this study. The results showed that fever onset as defined as a temperature $\geq 39^{\circ}\text{C}$ for at least 1 hour correlated with a temperature elevation $\geq 1.5^{\circ}\text{C}$ above the average baseline for 2 hours (Figure 12). These observations were similar to that observed in the natural history studies.

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Figure 12 : Baseline and Post-Challenge Temperature tracings for cohort 1

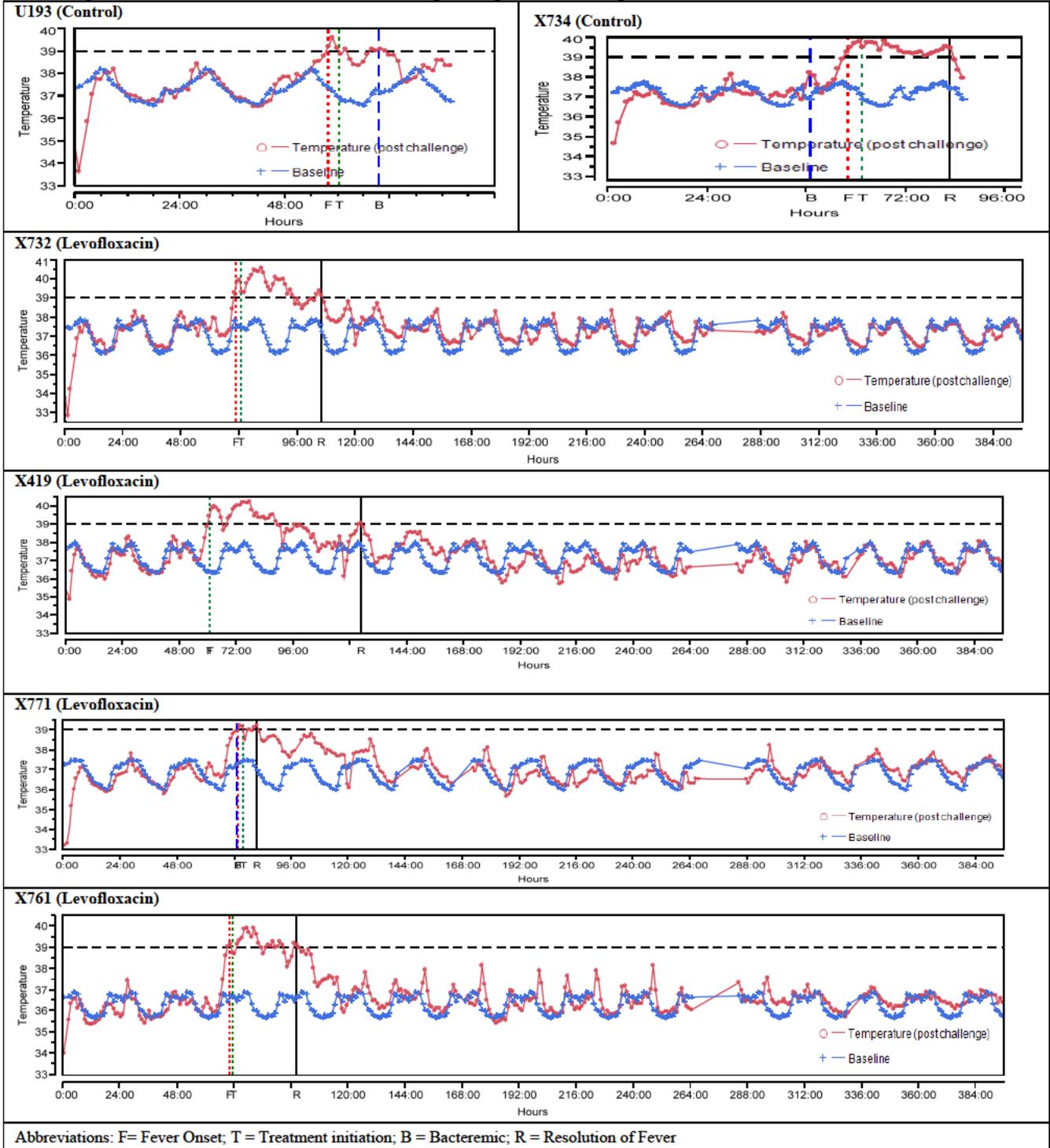


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Figure 12cont'd: Baseline and Post-Challenge Temperature tracings for cohort 2

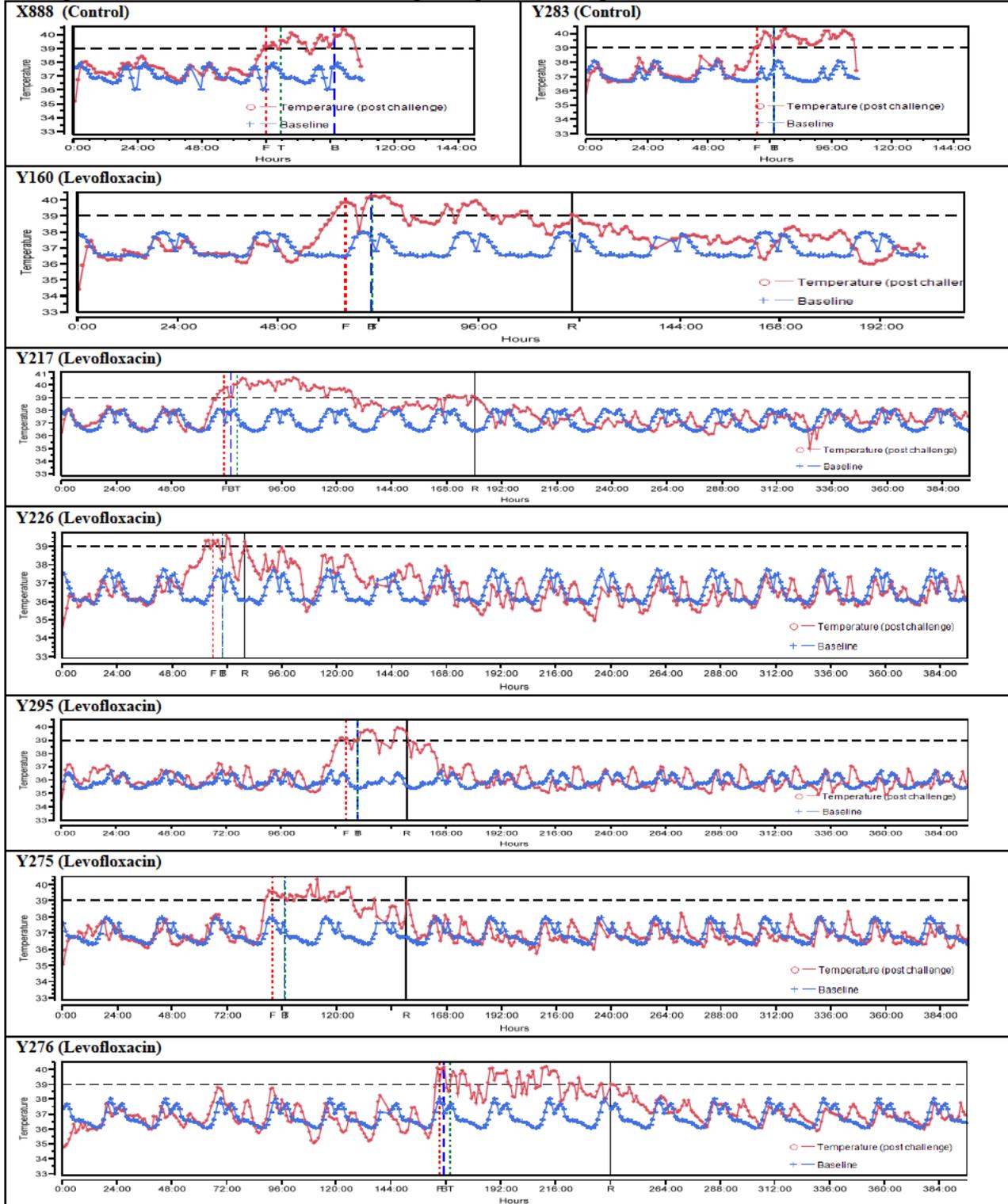


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Figure 12cont'd: Baseline and Post-Challenge Temperature tracings for cohort 3



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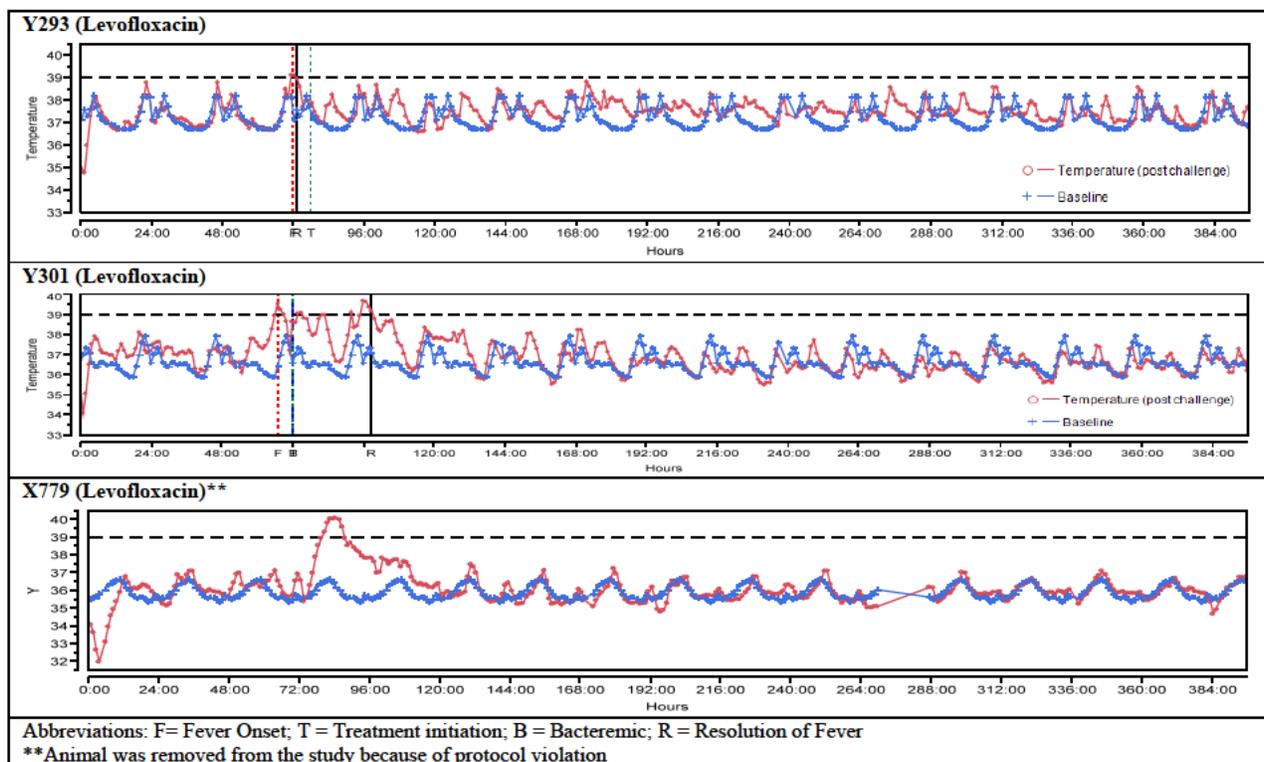
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Treatment

All animals were treated on the basis of fever response. Animals were administered levofloxacin approximately 1 to 6 hours after fever onset. All animals received the full 10 days (20 doses) of levofloxacin treatment with the exception of:

- Animal #Y160 received only 6 treatments (total of 12 infusions) and was euthanized on day 9 post-exposure due to a gastric complication unrelated to the plague infection.
- Animal #X779 received 1 full infusion dose of levofloxacin prior to becoming febrile. At 3 days post challenge, the animal received a single administration of 8 mg/kg levofloxacin. This animal was removed from the study due to a protocol deviation since the animal did not become febrile until approximately 7 hours later. Upon further investigation of this animal, it was also noted by the applicant (see *Submission dated March 12, 2012*) that the animal received an additional partial levofloxacin dose (0.5 mL of a 6.29 mL of the 5 mg/kg levofloxacin) at 9 days post challenge. The animal survived to May 30, 2008, at which time the animal was euthanized.

Bacteremia

Fourteen animals (3 controls and 11 treated) had blood and/or tissue samples that were contaminated with organisms other than *Y. pestis* throughout the study (Table 28 and Table

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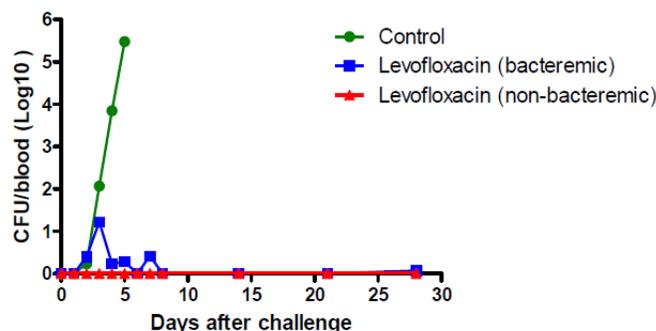
29). The investigator categorizes these contaminants as a procedural problem resulting in low-level contamination of non *Y. pestis* organisms due to:

- catheter biofilm formation in animals # X702, X773, X663, X523, Y295, Y275, Y276, Y293
- catheter tips in animals #Y295, Y275, Y276, Y293 and Y301 (The catheter tips were cultured only from animals in cohort 3. Cohorts 1 and 2 animals did not have the catheter tips cultured).
- improper handling of tissue samples in animals #X771, Y217, Y295
- excessive condensation onto the plates after refrigerator storage in animals #X648, X437, X761

Animals with catheter blood samples contaminated had bacterial concentrations to the magnitude of 3,000 to >300,000 CFU/mL. The identification of the bacterial contaminant(s) was not performed. The applicant determined that the animals were free of any secondary bacterial infection because the animals had subsequent blood cultures and tissues samples at later time points that were negative for microbial growth other than *Y. pestis*.

Viable *Y. pestis* colonies were observed starting at day-2 post-challenge in the levofloxacin and control animals (Figure 13). The starting bacterial titers ranged from 13 CFU/mL to 71,700 CFU/mL, similar to the titers observed in the natural history studies (Table 28). Control animals remained positive during the course and at the time of death. The terminal titer levels for the 7 controls ranged from 11,481 to 301,995 CFU/mL.

Figure 13: Average CFU in the blood of levofloxacin treated and control AGMs challenged with *Y. pestis* CO92 strain



In the treatment group, 5 animals (#X663, X419, X732, X761, Y293 and X779) were abacteremic prior to or at the time of antibiotic treatment (Figure 12).

- Animal Y293 had a temperature >39°C for at least 2 hours starting at 73 hours post-challenge. Blood culture samples at this time grew 1 colony of *Y. pestis* (1 plate); all other blood plates (6 plates) were contaminated with colonies non-*Y. pestis* organisms; the identity of the bacterial contaminant was not performed.

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- Animal X779, which was taken out of the study; the applicant stated that blood samples were collected on day 2, day 3, day 4, day 14, day 21 and day 28 (see submission dated *March 12, 2012*). All blood samples taken at each of the specified time points from this animal were negative for *Y. pestis*. The applicant stated that clinical observations of the animal at the time the animal was febrile (days 3 – 10 post challenge) included reduced/anorexic appetite, rough hair coat appearance and hunched posture.

The remaining 12 animals were bacteremic at the time of initiation of antibiotic treatment including the animals in cohort 3 with $<20LD_{50}$:

- Animal #Y226 had starting bacterial titers of 226 CFU/mL on day 3
- Animal #Y295 bacterial titer was 33 CFU/mL on day 5
- Animal #Y275 bacterial titer was 923 CFU/mL on day 4
- Animal #Y276 bacterial titer was 4,930 CFU/mL on day 7
- Animal #Y301 bacterial titer was 372 CFU/mL on day 3
- Animal #Y160 had starting bacterial titers of 5,000 CFU/mL on day 3.

All animals that received a challenge dose $<20LD_{50}$, in addition to becoming bacteremic, responded to treatment with levofloxacin and survived to Day 28.

All of the levofloxacin treated animals had bacterial levels below detectable limits by the 2nd day of treatment and had terminal cultures that were negative for *Y. pestis* with the exception of:

- Animal #648 had starting bacterial titers of 87 CFU/mL at the start of treatment which was below detectable levels by the 2nd day of treatment (Day 4 post-challenge). This animal received the full 10-day therapy (20 infusions); however had a terminal blood culture titer of 6.67 CFU/mL.
- Animal #663 was not positive for *Y. pestis* at the time of or during treatment but had terminal blood cultures on Day 28 positive for *Y. pestis* (3 CFU/mL).

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Table 28: Blood Culture Results for the 24 AGMs challenged with *Y. pestis* CO92 strain

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 ^{20Q}	BLD ^{>3.0e+05}	BLD	9.03E+03	>3.0E+05						
1	Control	X773	BLD ^{>3.0e+03}	BLD ^{>2.0e+05}	BLD	1.13E+02	1.14E+04						
1	Control	X762	BLD	BLD	7.17E+04	2.82E+04	>3.0E+05						
1	Levofloxacin	X663	BLD ^{>3.0e+03}	BLD ^{>3.0e+05}	BLD	BLD	BLD	BLD	BLD		BLD	BLD	3.33E+00
1	Levofloxacin	X662	BLD	BLD	5.67E+01	BLD	BLD	BLD	BLD		BLD	BLD	BLD
1	Levofloxacin	X648	BLD	BLD	8.67E+01	BLD	BLD	BLD	BLD		BLD ^{3.3e+00}	BLD	6.67E+00
1	Levofloxacin	X437	BLD	BLD	BLD	BLD	2.27E+03	BLD	BLD	BLD	BLD ^{1.2e+02}	BLD	BLD
1	Levofloxacin	X523	BLD	6.23E+04	BLD	BLD	BLD	BLD	BLD		BLD	BLD ^{>3.0e+03}	BLD ^{>3.0e+03}
2	Control	U193	BLD	BLD	2.97E+02	2.96E+05							
2	Control	X734	BLD	1.33E+01	3.90E+04								
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	8.03E+02	BLD	BLD	BLD			BLD	BLD	BLD
2	Levofloxacin	X761	BLD	BLD	BLD	BLD	BLD	BLD			BLD	BLD	BLD
3	Control	X888	BLD	BLD	BLD	1.51E+04							NS
3	Control	Y283	BLD	BLD	3.87E+02	BLD ^{3.77E+04}	>3.0E+05						
3	Levofloxacin	Y160	BLD	BLD	5.00E+03	BLD	BLD	BLD	BLD	BLD			
3	Levofloxacin	Y217	BLD	BLD	1.83E+02	BLD	BLD	BLD	BLD		BLD	BLD	BLD
3	Levofloxacin	Y226	BLD	BLD	1.76E+02	BLD	BLD	BLD	BLD		BLD	BLD	BLD
3	Levofloxacin	Y295	BLD	BLD	BLD	BLD	3.33E+01	BLD	BLD		BLD	BLD	BLD
3	Levofloxacin	Y275	BLD	BLD	BLD	9.23E+02	BLD	BLD	BLD	BLD	BLD	BLD	BLD
3	Levofloxacin	Y276	BLD	BLD	BLD	BLD	BLD	BLD	4.93E+04	BLD	BLD	BLD	BLD
3	Levofloxacin	Y293	BLD	BLD	BLD	BLD	BLD	BLD	BLD		BLD	BLD	BLD
3	Levofloxacin	Y301	BLD	BLD	3.27E+02	BLD	BLD	BLD	BLD		BLD	BLD	BLD

Abbreviations: BLD = Below limit of detection (negative culture), BLD (with superscript) = bacterial contaminant (not *Y. pestis*) colony count, if performed
Colors: Green highlight = bacterial contaminant (not *Y. pestis*); Pink highlight = Animals that were not bacteremic; Orange highlight = Animals with challenge dose < 20LD₅₀

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Cultures were performed on select tissues obtained from the spleen, liver, tracheobronchial lymph node and lung (lesion and non-lesion areas). Control animal tissue burden was particularly high for *Y. pestis* organisms in the lung especially from lesion areas (range, 10^7 – 10^9 CFU/g/tissue). A bacterial load in the range of 10^5 to 10^8 CFU/g was reported in tissues from the tracheobronchial lymph nodes, liver and spleen. Levofloxacin treated animals were negative for *Y. pestis* in tissue culture (only lung tissue cultures were evaluated) with the exception of:

- Animal #X648 had positive lung tissue (lesion) and a terminal positive blood culture for *Y. pestis*.
- Animal #X523 only had a positive lung culture but negative blood culture.

Table 29: Tissue Culture Results for the 24 AGMs challenged with *Y. pestis* CO92 strain

Cohort	Treatment	Animal ID	Spleen	Liver	TBLN	Lung - NL	Lung - L
1	Control	X702	3.24E+07	3.93E+08	8.78E+09	3.03E+09	2.43E+09
1	Control	X773	4.47E+06	8.32E+06	3.52E+08	4.89E+08	6.53E+09
1	Control	X762	3.00E+07	1.86E+08	3.09E+09	3.79E+08	9.61E+09
1	Levofloxacin	X663				BLD	BLD
1	Levofloxacin	X662				BLD	BLD
1	Levofloxacin	X648				BLD ^{5.3e+02}	1.77E+02
1	Levofloxacin	X437				BLD	BLD
1	Levofloxacin	X523				3.02E+02	BLD
2	Control	U193	6.86E+07	1.11E+08	1.68E+09	2.81E+07	1.53E+10
2	Control	X734	2.04E+09	6.28E+08	2.07E+09	4.29E+08	1.05E+10
2	Levofloxacin	X732				BLD	BLD
2	Levofloxacin	X419				BLD	BLD
2	Levofloxacin	X771				BLD	BLD ^{-6.8e+04}
2	Levofloxacin	X761				BLD	BLD
3	Control	X888	8.82E+09	2.32E+08	7.47E+09	1.46E+09	1.44E+10
3	Control	Y283	2.50E+09	1.74E+08	4.85E+09	5.69E+09	4.39E+09
3	Levofloxacin	Y160	BLD	BLD	BLD	BLD	BLD
3	Levofloxacin	Y217				BLD	BLD
3	Levofloxacin	Y226				BLD	BLD
3	Levofloxacin	Y295				BLD	BLD
3	Levofloxacin	Y275				BLD	BLD
3	Levofloxacin	Y276				BLD	BLD
3	Levofloxacin	Y293	BLD	BLD	BLD	BLD	BLD
3	Levofloxacin	Y301				BLD	BLD

Abbreviations: BLD = Below limit of detection, BLD (with superscript) = bacterial contaminant (not *Y. pestis*) colony count, if performed; TBLN = Tracheobronchial lymph node; Lung-NL = Lung Non-Lesion; Lung-L = Lung Lesion

Colors: Green highlight = bacterial contaminant (not *Y. pestis*); Pink highlight = Animals that were not bacteremic; Orange highlight = Animals with challenge dose < 20LD₅₀

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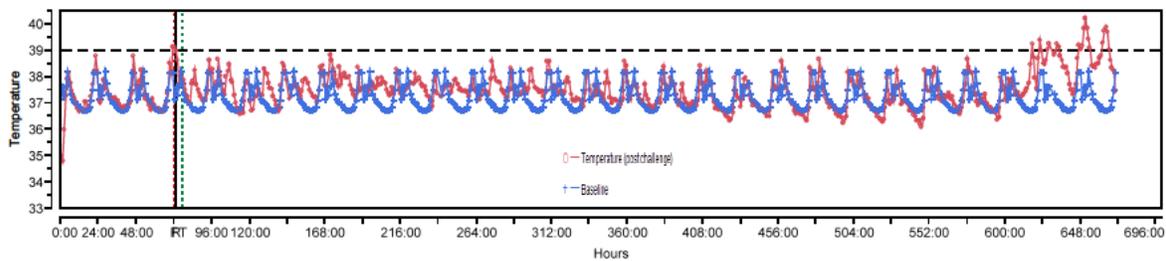
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Fever Resolution

Control animals had elevated temperatures prior to death (Figure 12). In the levofloxacin treated animals, fever resolved on average 35 hours post-treatment (range, 6 - 103 hours) with the exception of:

- Animal #Y293 had resolution of fever within one hour after fever onset and treatment (Figure 14). However, the animal's temperature increased to $>39^{\circ}\text{C}$ starting on day 26 and continued through Day 28. The animal's blood cultures taken from the catheter were negative for *Y. pestis* at all sample time points following levofloxacin administration. Upon termination of the study, terminal blood samples showed bacterial concentrations greater than 300 CFU/mL, not identified as *Y. pestis*, related to a contaminated catheter. The identification of the contaminant was not performed.

Figure 14: Temperature Tracings for Animal #Y293 in Cohort 3



Clinical Observations

Thoracic radiographs were performed on all animals in the study prior to *Y. pestis* challenge. All animals showed clear lung fields (Table 30). Follow-up radiographs were only performed on animals in cohorts 1 and 2 at Day 5/6 post-challenge to determine if animals showed radiographic evidence of pulmonary infiltrates. Control animals had pulmonary infiltrates which were characterized as severe involving both lungs. In comparison, the levofloxacin treated animals had bilateral infiltrates; however, these infiltrates were characterized as mild in 3 animals (#X663, X437 and X761) or moderate in 6 animals (#X523, X732, X449, X771, X648 and Y160). Six of these 9 animals had normal chest x-rays on follow-up at the end of the study.

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Table 30 : Summary of Radiographic Infiltrates observed in the lungs of AGMs challenged with *Y. pestis* CO92 strain

Animal ID	Cohort	Treatment	Baseline	Day 5, 6 or 9*	Day 28
X702	1	Control	N	+++	--
X773	1	Control	N	+++	--
X762	1	Control	N	+++	--
X663	1	Levofloxacin	N	+	N
X662	1	Levofloxacin	N	+	--
X648	1	Levofloxacin	N	++	--
X437	1	Levofloxacin	N	+	--
X523	1	Levofloxacin	N	++	N
U193	2	Control	N	--	--
X734	2	Control	N	--	--
X732	2	Levofloxacin	N	++	N
X419	2	Levofloxacin	N	++	N
X771	2	Levofloxacin	N	++	N
X761	2	Levofloxacin	N	+	N
X888	3	Control	N	--	--
Y283	3	Control	N	--	--
Y160	3	Levofloxacin	N	++	--
Y217	3	Levofloxacin	N	--	--
Y226	3	Levofloxacin	N	--	--
Y295	3	Levofloxacin	N	--	--
Y275	3	Levofloxacin	N	--	--
Y276	3	Levofloxacin	N	--	--
Y293	3	Levofloxacin	N	--	--
Y301	3	Levofloxacin	N	--	--

*Radiographs were performed on Day 6 instead of Day 4 for animals# X437, X523, X648, X662, X663

*Animal Y160 had radiograph performed on Day 9 due to gastric complications

Pulmonary infiltrates defined as N= normal; += mild; ++ = moderate; +++ = severe; dashes = not done

Pharmacokinetics/Pharmacodynamics

Plasma drug concentrations of peak and trough levels were monitored in the levofloxacin-treated animals. However, the results of the treated animals from Cohort 1 and 2 were not interpretable. For cohort 3, the peak (C_{max}) concentration of levofloxacin after the first and third doses of 8 mg/kg ranged from 2.4 to 4.5 $\mu\text{g/mL}$ (Table 31). The trough level for levofloxacin ranged from 0.052 to 0.117 $\mu\text{g/mL}$. Peak levels of levofloxacin observed throughout the study were below the levels observed in humans administered as single or multiple i.v. doses of 500 mg ($C_{max} = 6.2 - 6.4 \mu\text{g/mL}$).

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There was no susceptibility testing performed on the isolates in the study. However, based on *in vitro* data from published studies the MIC value for levofloxacin against *Y. pestis* isolates ranges from <0.03 to 0.012 µg/mL. These MICs are at or below the trough values observed for levofloxacin in the study. Overall, the animals survived when treated with the full 10-day (20 doses) of levofloxacin at dosing that produced plasma levels in AGMs that were lower than the corresponding values seen in humans with a 500 mg IV dose of levofloxacin.

Table 31: Plasma Levofloxacin Levels for cohort 3

Infusion No.:	1 post		3 pre		3 post		4 post	
	8 mg/kg		2 mg/kg		8 mg/kg		2 mg/kg	
Dosage ^a :	8 mg/kg		2 mg/kg		8 mg/kg		2 mg/kg	
ID	Levo ^b	ΔT ^c	Levo	ΔT	Levo	ΔT	Levo	ΔT
Y160	2893.91	0:05	52.85	10:45	3016.46	0:08	1015.10	0:05
Y217	4514.94	0:06	60.50	10:50	3841.48	0:05	1304.52	0:05
Y226	3227.63	0:05	59.51	10:47	2475.65	0:09	1009.28	0:06
Y295	2428.36	0:10	35.53	11:16	3426.33	0:08	724.13	0:05
Y275	2582.17	0:05	117.33	10:23	3320.44	0:05	1445.88	0:05
Y276	4260.60	0:06	70.02	11:08	2909.78	0:05	1102.96	0:05
Y293	2688.83	0:05	95.97	10:49	2853.31	0:07	1084.79	0:07
Y301	4011.95	0:06	69.84	10:38	4552.10	0:05	945.10	0:08

^a Dosage level of previous infusion prior to blood collection

^b Plasma levofloxacin concentration (ng/mL)

^c Change in time (h:m) from end of previous infusion

Pathology

Gross necropsies and histopathologic evaluation were performed on all animals in the study:

Control animals (n = 7) were noted to have large deep purple regions in multiple lobes of the lungs, consistent with extensive parenchymal hemorrhage on gross necropsy. The tracheobronchial lymph nodes were characterized as enlarged and or discolored. Both the study and independent pathologists noted that the pathology in the controls was consistent of a pneumonic plague similar to that observed in the natural history studies. Overall, morphologic changes in the lung of controls appear to begin as lobar to sub-lobar serous and fibrinous exudates (edema) with the presence of intra-alveolar inflammatory infiltrates primarily neutrophils and/or macrophages (Table 32). In some animals edema, presence of macrophages and bacteria predominated in sections of the lung which appear to represent earlier lesions in the disease process. Large numbers of neutrophils were noted in affected areas along with hemorrhage and edema which eventually totally efface the normal lung tissue elements. Bacteria were observed in the lung and in both alveolar spaces and alveolar macrophages. Changes in the lymphoid organs were similar to that observed in the natural history studies. The tracheobronchial lymph nodes were characterized as having

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bacterial colonization, edema, hemorrhage, inflammatory infiltrates (primarily neutrophils) and lymphoid hyperplasia or depletion. Animals had one or more characteristic changes in the spleen which included presence of bacteria, inflammatory infiltrates (neutrophils), hemorrhage and lymphoid depletion. Bacteria were noted in blood vessels in one or more of the organs and tissues. Four animals were noted to have bacteria or inflammatory and cellular infiltrates (neutrophils, lymphoplasmacytic) in the liver. No marked changes were noted in the brain and heart of the control animals.

Table 32: Microscopic findings in the lungs and lymphoid organs of controls and treated animals

Tissue/Observation	Placebo (n = 7)	Levofloxacin Treated (n = 17)
Lung		
Bacteria	7	0
Cellular debris, bronchiolar	0	1
Edema	7	0
Hemorrhage	0	0
Inflammation, chronic, perivascular	0	13
Inflammatory infiltrate, interstitial/bronchiolar mononuclear cell	0	16
Inflammatory infiltrate, intraalveolar, macrophage	6	0
Inflammatory infiltrate, intraalveolar, neutrophils	7	0
Pleura, fibrin	4	8
Thrombus, organizing	0	1
Within normal limits	0	1
Lymph Node, bronchial/tracheobronchial		
Bacteria	7	0
Edema	5	0
Hemorrhage	2	0
Inflammatory infiltrate, neutrophils	4	1
Lymphoid depletion	4	0
Lymphoid Hyperplasia	0	1
Within normal limits	0	15
Spleen		
Bacteria	5	0
Congestion	1	0
Hemorrhage	1	0
Inflammatory infiltrate, macrophage	2	1
Inflammatory infiltrate, neutrophils	2	0
Lymphoid hyperplasia	0	1
Lymphoid depletion	3	1
Within normal limits	1	15
Brain		
Meninges, bacteria	0	0
Meninges, inflammatory infiltrate, lymphocyte	0	1
Within normal limits	7	16
Liver		
Bacteria	2	0
Inflammatory infiltrate, neutrophil	1	0
Periportal, cellular infiltrate, lymphoplasmacytic	2	2
Perivascular, inflammation, chronic	0	1
Sinusoid, cellular infiltrate, neutrophils	1	0
Within normal limits	3	15

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In the levofloxacin treated animals (n=17), in general, the lungs appearance on gross necropsy were characterized as having a discolored surface consistent with resolution of plague pneumonia or anesthesia. On histopathology evaluation, the changes in the lungs were noted to reflect resolution of the lesions of an initial pulmonary infection and characterized as chronic compared to the control animals. Inflammatory cells were noted in the interstitial or perivascular areas which consisted of mononuclear cells rather than neutrophils and macrophages. Pulmonary changes such as edema and hemorrhage were not present in the treated animals as observed in control animals. Bacteria were not observed in the alveoli or macrophages in the sections of the lungs. The tracheobronchial lymph nodes and spleen were characterized as normal, with the exception of some animals in which inflammatory infiltrates (animal #Y160) and lymphoid hyperplasia (animal #X771) were noted. Two animals (#X523 and Y293) had minimal chronic perivascular inflammation and cellular infiltrates primarily lymphoplasmacytic in the liver. One animal (#Y276) was noted to have a minimal lymphocytic infiltrate in the meninges, though the significance of these findings is unknown.

Animal Y160, a levofloxacin treated animal, was euthanized on day 9 post-challenge due to gastric complications. On gross examination, a lesion was noted on the stomach. Microscopic evaluation of lung tissue sections revealed resolving pulmonary lesions similar to those described for other treated animals. Histopathologic evaluation of the gastric epithelium was characterized as necrotic, which showed no evidence of the bacteria in the stomach. Select tissues from the spleen, liver, tracheobronchial lymph node and lung (lesion and non-lesion areas) collected at necropsy were negative for culture; suggesting that the death appear to be not related to *Y. pestis* challenge. However, the exact cause of the complication in this animal remains unexplained.

Outcome

The primary efficacy outcome was survival at the end of the study. The applicant determined results showed that the proportion of animals surviving in the levofloxacin treated group (16/17 or 94%) was significantly higher than the proportion of animals surviving in the control group (0/7 or 0%; $p < 0.001$). This analysis was based on animals that received the full treatment and control animals that received only placebo.

A number of sensitivity analyses for efficacy were performed on the survival endpoint as well as microbiologic response as follows:

- *Intention-to-treat*: The 26 AGMs allocated to treatment groups planned at randomization were included in the analyses. Overall, if the 2 animals (#X717 and X779) that was removed from the study were included the analyses as survivors or non-survivors, the results remained highly significant.

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Table 33: Clinical and Microbiological response of the levofloxacin –treated and control AGMs exposed to *Y. pestis* CO92

	Levofloxacin ^a			Placebo (saline)			95% CI ^b	p-value
	N	n	(%)	N	n	(%)		
<i>Survival</i>								
Intent to treat (ITT) ^{c,e,f}	17	16	(89)	7	0	(0)	(42.1, 98.6)	<0.0001
Cohort 1 and 2	9	9	(100)	5	0	(0)	(47.4%, 100%)	0.0005
Challenge Dose	11	11	(100)	7	0	(0)	(58.9, 100)	<0.0001
Bacteremic	12	11	(92)	5	0	(0)	(28.0, 99.8)	0.001
Radiographic Evidence	9	9	(100)	3	0	(0)	(29.0, 100)	0.005
<i>Microbiological Response</i>								
Microbiologic	17	14	(83)	7	0	(0)	(29.0, 96.3)	0.0003

^aLevofloxacin 8 mg/kg (high dose) levofloxacin followed by a second infusion within 12 hours of 2 mg/kg levofloxacin (low dose); Control animals received 5% dextrose

^bExact 95% Confidence Interval for difference in survival proportion was calculated using Fisher's exact Test (2-sided)

^cApplicant ITT population

^dIf X717 was included as non-survivor and X779 was included as survivor, 95% CI [42.1%, 98.7%], p-value <0.0001

^eIf both included as non-survivors, 95% CI [42.1%, 96.5%], p-value 0.0002

Note: N = the number of animals in each arm;

n = number of animals that were cured (microbiologically or clinically based on survival)

- Cohort 1 and 2:** Analyses of cohort 1 and 2 were performed since the applicant knew the results prior to conducting cohort 3. For animals in cohort 1 and 2, levofloxacin treated animals had a higher survival compared to placebo controls.
- Challenge Dose:** A cut-off of >20 LD₅₀ was chosen based on the natural history studies which showed that animals that received <20 LD₅₀ survived the challenge and showed no clinical signs and symptoms of disease. Analyses were conducted excluding the 6 levofloxacin-treated animals (Y160, Y226, Y295, Y275, Y276 and Y301) in Cohort 3 that received challenge doses ranging from 3 to 12 LD₅₀. Overall, the survival in animals that received >20LD₅₀ was higher in the levofloxacin treated group compared to placebo controls.
- Bacteremic at the time of treatment:** All animals that were bacteremic at the start of treatment were included in this analysis. There were 5 animals (#X663, X419, X732, X761 and Y293) in the levofloxacin treatment arm and 2 placebo controls (#X773 and X888) that did not have positive blood cultures for *Y. pestis* at the time of treatment. Among the animals that were bacteremic, the survival was higher in the levofloxacin treated group compared to placebo controls.
- Radiologic Evidence of Pulmonary infiltrates:** Animals in cohorts 1 and 2 had chest radiographs performed post-challenge to *Y. pestis* exposure. Animals in Cohort 3 had baseline radiographs performed but none at follow-up, these animals were excluded from the analysis. In addition, 2 control animals from cohort 1 and 2 died or were euthanized before chest radiographs could be performed were excluded. Overall, the survival was higher in the levofloxacin treated group compared to placebo controls.

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- *Microbiologic Response/Cure:* Analyses were performed on all animals that had cultures at study termination on Day 28 or at the time of death/euthanasia. All control animals were bacteremic at the time of euthanasia/death. Three levofloxacin treated animals (#X663, X648 and X523) had blood cultures and/or lung tissue positive for *Y. pestis* at study termination on Day 28. Two animals had positive blood cultures (#X663 and #X648) and two animals had positive tissue cultures (#X648 and #523). The remaining 13 levofloxacin treated animals resolved their bacteremia within 24 hours after first levofloxacin dose and had negative tissue cultures at study termination.

5. CLINICAL STUDIES

A pubmed search of the literature showed no reported cases in which levofloxacin was used in the U.S. for the treatment of human cases of natural plague.

6. DISCUSSION

A single study was performed in African Green monkeys that evaluated the efficacy of levofloxacin to support a treatment indication for inhalation plague in adults and pediatric patient (>50 kg and \geq 6 months of age). A total 25 animals were challenged via aerosol route to the *Y. pestis* CO92 strain with a target dose of 100 ± 50 LD₅₀. The challenge was performed in 3 iterations consisting of 8 animals in cohort 1, 7 in cohort 2 and 10 animals in cohort 3. The corresponding LD₅₀ equivalent in all animals challenged ranged from 3 to 148 LD₅₀. Animals in cohort 3 received significantly fewer organisms (22.1 ± 23 LD₅₀) than animals in cohorts 1 and 2 (74.2 ± 31.03 LD₅₀ and 124.4 ± 10.5 LD₅₀, respectively). Six levofloxacin treated animals from cohort 3 (#Y160, Y226, Y295, Y275, Y276 and Y301) had aerosol exposures that ranged from 3 to 12 LD₅₀. The trigger for treatment was onset of fever defined as an increase in body temperature $\geq 39^\circ\text{C}$ for at least 1 hour post-challenge. At fever onset, animals were randomized to treatment of 8 mg/kg (high dose) levofloxacin followed by a second infusion within 12 hours of 2 mg/kg (low dose) levofloxacin for 10 days. Control animals were administered similar volumes of 5% dextrose for 10 days.

All control animals (n = 7) had signs and symptoms of clinical disease within 2 – 4 days post-challenge (Table 34). The onset of fever ranged from 57 to 93 hours post-challenge. Control animals were bacteremic at fever onset and succumbed to the infection within 105.3 ± 14.5 hours post-challenge. Four were found dead (#X702, X734, X888 and Y283) and 3 animals (#X773, X762 and U193) were euthanized due to moribund condition. Terminal bacterial blood counts for the controls ranged from 11,481 to 301,995 CFU/mL. Similar to the animals in the natural history studies, gross pathology and histopathology findings in the control animals were consistent with pneumonic plague. The gross pathology data supported the clinical data in that bacteria consistent with *Y. pestis* were observed in the lungs of controls. Numerous intra-alveolar bacteria, alveolar flooding and

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hematogenous spread of the organism to other organs such as the spleen and lymph nodes showing the presence of inflammatory infiltrates (primarily neutrophils) were noted. Tissue samples of the lungs, tracheobronchial lymph nodes, spleen, liver and blood showed an overwhelming presence of *Y. pestis* bacteria in the order of $>10^6$ CFU/g tissue.

Table 34: Summary of 17 levofloxacin treated and 7 control AGMs exposed to aerosols of the *Y. pestis* CO92 strain

Challenge Days	Animal ID	Treatment	Estimated Inhaled Dose (CFU)	LD ₅₀ equivalents	Fever Onset [†] (hours)	Time to Bacteremia (hours)	Time to Treatment (hours)	Time to Death (hours)	Outcome
1	X702	Control	1.95 x 10 ⁴	56	93	93	94	118	FD
1	X773	Control	5.02 x 10 ⁴	143	65	93	69	113	EU
1	X762	Control	2.65 x 10 ⁴	76	74	70	75	121	EU
1	X663	Levofloxacin	2.32 x 10 ⁴	66	59	NB	60	>660	S
1	X662	Levofloxacin	2.82 x 10 ⁴	81	67	68	68	>660	S
1	X648	Levofloxacin	1.99 x 10 ⁴	57	70	80	72	>660	S
1	X437	Levofloxacin	1.39 x 10 ⁴	40	123	121	125	>660	S
1	X523	Levofloxacin	2.63 x 10 ⁴	75	52	46	57	>660	S
2	U193	Control	4.25 x 10 ⁴	121	58	69	60	85	EU
2	X734	Control	5.08 x 10 ⁴	145	57	49	61	86	FD
2	X732	Levofloxacin	4.32 x 10 ⁴	124	70	NB	72	>660	S
2	X419	Levofloxacin	4.20 x 10 ⁴	120	60	NB	66	>660	S
2	X771	Levofloxacin	4.13 x 10 ⁴	118	74	73	75	>660	S
2	X761	Levofloxacin	4.13 x 10 ⁴	118	69	NB	71	>660	S
3	X888	Control	1.55 x 10 ⁴	44	73	97	77	109	FD
3	Y283	Control	1.66 x 10 ⁴	47	68	73	73	105	FD
3	Y160	Levofloxacin	2.11 x 10 ³	6	64	70	70	203	EU
3	Y217	Levofloxacin	1.32 x 10 ⁴	38	71	73	76	>660	S
3	Y226	Levofloxacin	4.21 x 10 ³	12	66	70	70	>660	S
3	Y295	Levofloxacin	1.04 x 10 ³	3	124	129	129	>660	S
3	Y275	Levofloxacin	1.25 x 10 ³	4	92	97	97	>660	S
3	Y276	Levofloxacin	9.20 x 10 ²	3	165	167	170	>660	S
3	Y293	Levofloxacin	2.18 x 10 ⁴	62	73	NB	77	>660	S
3	Y301	Levofloxacin	9.25 x 10 ²	3	67	71	72	>660	S

Of the remaining 17 levofloxacin treated animals, 16 survived the *Y. pestis* challenge (Table 34). Similar to the control animals and animals in the natural history studies, the time interval from exposure to *Y. pestis* aerosol to fever onset ranged from 52 to 165 hours post challenge. All animals were treated with levofloxacin approximately 1 to 6 hours after fever onset. All animals received the full 10 days (20 doses) of levofloxacin. For the following:

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- animal #Y160 received only 6 treatments and was euthanized on day 9 post-exposure due to a gastric complication unrelated to the plague infection.
- Animal #X779 received a single infusion of levofloxacin prior to manifesting a fever of $> 39^{\circ}\text{C}$ for 1 hour; this animal was removed from the study. This animal was removed from the study due to a protocol deviation since the animal was treated 7 hours prior to the development of fever. The animal survived to Day 28 post-challenge at which time the animal was euthanized. No further information was provided on this animal.
- Five animals (#X663, X419, X732, X761 and Y293) were abacteremic prior to or at the time of antibiotic treatment
- Animal #648 had starting bacterial counts of 87 CFU/mL at the start of treatment that was below detectable levels by the 2nd day of treatment (Day 4 post-challenge). This animal received the full 10-day therapy (20 infusions), however had a terminal blood (6.67 CFU/mL) and tissue culture (177 CFU/g) positive for *Y. pestis*.
- Animal #663 was not positive for *Y. pestis* at the time of or during treatment but had terminal blood cultures on Day 28 positive for *Y. pestis* (3 CFU/mL).
- Animal #X523 only had a positive lung culture (302 CFU/g) on Day 28 positive for *Y. pestis*.
- Animal #Y293 which had resolution of fever within one hour after fever onset and treatment (Figure 14). However, the animal's temperature increased to $>39^{\circ}\text{C}$ starting on day 26 through to the end of the study. This animal received a challenge dose of 62 LD₅₀ *Y. pestis* CO92 strain. The animal's blood cultures taken from the catheter were negative for *Y. pestis* at all sample time points prior to and following levofloxacin administration. Terminal blood samples showed bacterial concentrations greater than 300 CFU/mL, not identified as *Y. pestis*, related to a contaminated catheter. The identification of the contaminant was not performed.

Overall, the proportion of animals surviving in the levofloxacin treated group (94%) was significantly higher than the proportion of animals surviving in the control group (0%). The proportion of animals that survived in the levofloxacin group remained significantly higher than control animals when sensitivity analyses were performed based on study cohort, challenge dose, animals that were bacteremic at the start of treatment and radiologic evidence of pulmonary infiltrates. Even though 3 animals in the levofloxacin treatment group had positive blood and/or tissue cultures at study termination on Day 28 which were considered microbiological failures. The bacteremia in the remaining levofloxacin treated animals resolved within 24 hours after first levofloxacin dose.

7. LABELING

The *Microbiology* subsection of the package should be revised as follows:

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(1) *Yersinia pestis* should be added to the *in vitro* activity listing (list #2)

(2) The applicant suggests that the following statement should be added:

(b) (4)

Since the above statement will be included in the *Indications* section of the labeling, it is not necessary to include in the Microbiology section.

(3) For Table 11 of the package insert, a susceptibility breakpoint of ≤ 0.25 $\mu\text{g/mL}$ should be provided for *Yersinia pestis*

The *Microbiology* subsection of the package insert should read as follows (Additions are noted by a double underline and deletions are noted by strikethrough):

(b) (4)

3 pages of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

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(b) (4)

15. REFERENCES

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2. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; 22nd Informational Supplement. CLSI Document M100-S22, 2012.
3. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard – 11th Ed ed. CLSI Document M02-A11, 2012.

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4. CLSI. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline – 2nd ed. CLSI Document M45-A2, 2010.

8. RECOMMENDATIONS

The efficacy supplement is approvable with respect to microbiology pending an accepted version of the labeling.

9. REFERENCES

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April 4, 2012

Simone M. Shurland, Ph.D.
Clinical Microbiology Reviewer, DAIP

5 Apr 12 FIN FJM

Frederic J. Marsik, Ph.D., ABMM
Microbiology Team Leader DAIP

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

/s/

SIMONE SHURLAND
04/05/2012

FREDERIC J MARSIK
04/05/2012

MICROBIOLOGY FILING CHECKLIST for Supplement Review

	Content Parameter	Yes	No	Comments
10	Has the applicant used standardized or nonstandardized methods for measuring microbiologic outcome? If nonstandardized methods were used, has the applicant included complete details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done?	X		
11	Has the applicant <u>submitted</u> draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package?	X		
12	Has the applicant <u>submitted</u> annotated microbiology draft labeling consistent with current divisional policy, and the design of the development package?	X		
13	Have all the study reports, published articles, and other references been included and cross-referenced in the annotated draft labeling or summary section of the submission?	X		
14	Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review?		X	

IS THE MICROBIOLOGY SECTION OF THE APPLICATION FILEABLE?

X YES NO

If the NDA is not fileable from the microbiology 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.

Simone M. Shurland, Ph.D.

1/3/2011

Reviewing Microbiologist

Date

Frederic J. Marsik, Ph.D. ABMM

1/3/2011

Microbiology Team Leader

Date

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/s/

SIMONE SHURLAND
01/03/2012

FREDERIC J MARSIK
01/03/2012

Division of Anti-Infective Products Clinical Microbiology Review

IND#: 36,627 (SDN-873,875), 38,368 (SDN-644, 646)

NDA#: 20-634 (SDN-485, 486) 20-635 (SDN-495, 496), 21-721 (SDN-199, 200)

Reviewer: Simone M. Shurland

Date Company Submitted: 4/29/2011, 5/20/2011

Date Received by CDER: 4/29/2011, 5/23/2011

Date Assigned: 4/29/2011, 5/25/2011

SPONSOR

Ortho-McNeil-Janssen Pharmaceutical, Inc.

Johnson & Johnson Pharmaceutical Research & Development, LLC

920 Route 202 South, PO Box 300

Raritan, NJ 08869-0602

DRUG PRODUCT NAME

Proprietary Name: Levaquin®

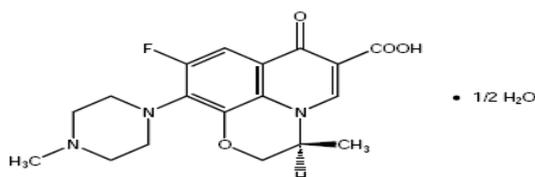
Non-Proprietary Name: Levofloxacin

Chemical Name: (-)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemihydrate

Molecular Weight: 370.38

Empirical Formula: C₁₆H₁₄ F₃N₅O

Structural Formula:



DRUG CATEGORY

Anti-bacterial

PROPOSED INDICATION

Treatment of plague

PROPOSED DOSAGE FORM, STRENGTH, ROUTE OF ADMINISTRATION AND DURATION OF TREATMENT

Dosage Form: Tablets

Strength: 250 mg, 500 mg, 750 mg

Route of Administration: Oral

DISPENSED

Prescription product (Rx)

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RELATED DOCUMENTS

IND# 64,429

SUMMARY AND RECOMMENDATIONS:

The sponsor has responded to the Division request to provide “mock up” electronic datasets for the efficacy and natural history studies. The following recommendations were communicated to the sponsor on June 6, 2011:

Questions for FDA based on May 10, 2011 response

FDA Recommendation: If contaminants are identified then these should be provided as well in the dataset; such information should include the species identified, the site/source (e.g., catheter) and bacterial counts (if available).

J&JRD Response: *Please clarify if these are to be included in the study reports or the SAS dataset for statistical analysis. It may be difficult to retrospectively put the data into a uniform format for a SAS dataset; it should be very straightforward to include them in the report(s) if the data are available.*

FDA response:

It is acceptable to provide the information in the study reports.

FDA Recommendation: We would like the different parameters such as for the aerosol and microbiology information in the datasets to be presented as columns for each animal rather than as rows.

J&JRD Response: *Please clarify whether this is the direction of all reviewers and not just the Microbiology reviewers. The guidance regarding CDISC format was followed for the mock dataset. We will submit a single format for each dataset, though we are willing to tailor dataset to column or row format. The data is almost never collected in a column per animal so it will require transformation in every case.*

FDA Response:

The content of the datasets is acceptable. With regard to the format of the datasets, enclosed please find sample datasets for hematology, telemetry, and bacteremia results. We suggest that:

- Chemistry results should be presented in a similar format to the hematology dataset, i.e. parameters should be presented as columns for each animal.
- Laboratory test results (hematology, chemistry, coagulation, blood gases) should be presented with normal ranges (upper and lower limit of normal).
- For aerosol and microbiology information, the different parameters should be presented as columns for each animal rather than as rows.

Additional Clinical comments

For the Data Listings and Data Tabulations:

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- a) There may be numerous data points for some measurements. For example, given that animals were monitored via telemetry, there may be thousands of data points for vital signs. We recommend submitting the following for each animal *in lieu* of the actual telemetry tracings:
- *Temperature* – from the time of the start of the study through the time of death of the animal, provide mean (standard deviation [SD] and range) hourly temperature per animal tabulated over time. This would therefore involve approximately 24 data points per day and 120 data points over 5 days. This will allow an assessment of trends and a clarification of the variation within the hourly temperature.
 - *Respiration* – provide mean (SD and range) hourly respiratory rates in a tabulated database, and clarify discrepancies between respiratory rates obtained by observation and by telemetry, if necessary.
 - *Heart Rate* – provide mean (SD and range) hourly heart rates in a tabulated database.
 - *Blood Pressure* – provide mean (SD and range) hourly systolic and diastolic blood pressure and pulse pressure.
 - *Please clarify what the variable “pressure” refers to in the table entitled “Telemetry Data Definitions for Study 875” submitted on May 20, 2011*
- b) In addition, we request all data on the following measurements in summary tabulations at all the time points when the measurements were done.
- Body weight
 - Inoculum size delivered via aerosol
 - Clinical observations - signs and or symptoms of illness: Provide tabulation of animal activity over the study period, documenting behavior, and appetite, and response to stimuli at each time point when observations were collected from baseline to euthanasia or death.
 - Blood cultures: *Y. pestis* CFU/mL for each animal at baseline, during treatment and follow-up including date and time when the samples were collected.
 - X-rays: Please include the interpretive findings by radiologist
 - Gross pathology (including organ culture results)
 - Histopathology - For histopathology data, individual animal reports and a summary table describing the specific findings (e.g., severity, extent and nature of histologic changes, utilizing a standard scale) in each organ examined.
 - Complete medical record or document that was used to collect data for each animal used in the study. The complete medical record should provide the information on everything that occurred to the animal prior to entry into the study (e.g., when anesthetized, any medications administered, prior infections, vaccinations, screening for pathogens, etc.). Indicate in the dataset whether the animals that were used are “experimentally naïve” or if the animals were previously used in any other experimental study(ies).

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INTRODUCTION

The subject of this IND is levofloxacin, a fluoroquinolone for the treatment of inhalational plague. Levofloxacin is approved for the treatment of Acute Maxillary Sinusitis, Acute Bacterial Exacerbation of Chronic Bronchitis, Community-Acquired Pneumonia, Complicated Urinary Tract Infections, Acute Pyelonephritis, Uncomplicated Skin and Skin Structure Infections, Uncomplicated Urinary Tract Infections, Complicated Skin, Skin Structure Infections and Nosocomial Pneumonia, MDRSP and Inhalational Anthrax.

BACKGROUND

On November 1, 2010, the sponsor provided a meeting background package which included a list of questions and pre-supplemental information for a future NDA submission of levofloxacin (NDA 20-634, NDA 20-635 and NDA 21-721) for the indication of pneumonic plague in adults and pediatric patients (>50 kg and ≥ 6 months of age). Animal efficacy studies and plague natural history studies to support the indication, sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), were conducted under IND 64,429. The Division's recommendations on the information provided were communicated in the letter dated November 30, 2010 as well as discussed at the type B meeting held on December 1, 2010 (see *FDA Teleconference Minutes* dated 2/7/2011).

The sponsor is responding to the Division request to provide sham data sets for the efficacy and natural history studies which was provided in the submission on April 29, 2011. The submission contained definitions and mock datasets for the plague natural history/efficacy supplement. More specifically, the microbiology team was asked to address the following question:

If the FDA is only interested in microbiology datasets from the efficacy study conducted in African Green Monkeys (FY07-070) then this would consist of bacterial counts from blood samples and target organs from individual monkeys in this study. If the FDA is interested in other microbiology datasets, then these could include (i) susceptibility data from MIC endpoint studies performed by USAMRIID and HPA in which collections of *Y. pestis* isolates were tested against Levofloxacin, and/or (ii) microbiology endpoint data as included in Natural History Studies [Battelle (617-G607610), Lovelace (FY06-126), and USAMRIID] which would include bacterial counts from blood samples and/ or target organs from individual monkeys in these studies. Note that no quantitative microbiology endpoint data were collected in the two NIAID-sponsored efficacy studies of Levofloxacin conducted in mice so the FDA request for microbiology datasets would not apply to these two studies.

The Division provided recommendations communicated in the letter dated May 10, 2011.

In this submission, the sponsor has responded to the Division recommendations provided on May 10, 2011.

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Response to FDA comments

The sponsor's responses to the specific Microbiology comments are discussed below (FDA comments are shown in bold and the sponsor's responses to FDA comments are discussed in regular font).

FDA Comment #1:

In addition to the microbiology datasets from the efficacy study (FY07-070) we recommend submitting the microbiology datasets for the

- **Natural History Studies conducted at:**
 - **Battelle (617-G607610),**
 - **Lovelace (FY06-126),**
 - **USAMRIID (F03-09G).**
- **If *in vitro* susceptibility testing was performed in the efficacy studies, then levofloxacin MICs against the *Yersinia pestis* isolates collected at the end of treatment should be included in the datasets.**

Sponsor's response:

The sponsor agreed.

FDA Comment #2

In reference to the sham datasets submitted on April 29, 2011, we recommend the following:

- **The microbiological culture results for the target organs (i.e., necropsy tissues) including the source, time point at which the sample was collected and bacterial colony counts.**

Sponsor's response:

The sponsor stated that the microbiology data will be added to the gross necropsy dataset. However, tissue microbiological data will only be available for natural history and control animals, since the survivors were not necropsied. The information will be updated to the data definitions file.

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FDA Comment:

- **If contaminants are identified then these should be provided as well in the dataset; such information should include the species identified, the site/source (e.g., catheter) and bacterial counts (if available).**

Sponsor's response:

The sponsor wanted further clarification whether the requested information could be included in the study reports rather than the SAS datasets for statistical analysis. The sponsor indicated the information may be more readily be incorporated in a study report (if the data are available) than incorporated into a SAS dataset format,

Reviewer comment:

It would be acceptable to provide the information requested in the study reports.

FDA Comment:

- **The different parameters such as for the aerosol and microbiology information in the datasets be presented as columns for each animal rather than as rows.**

Sponsor's response:

The sponsor wanted further clarification whether the above request is unanimous for all reviewers. The sponsor indicated the assembly of the datasets will be following CDISC format, in which each data point is represented by a row in the SAS data set. The sponsor indicated that each of the laboratories performing the studies that the raw data is also filed for each animal, with rows for time points and columns for the parameters (temperature, heart rate etc.). Following the CDISC format, the data is never collected in a column per animal, which will require transformation in every case. However, the sponsor is willing to tailor dataset to column or row format, if needed.

Reviewer comment:

With regard to the format of the datasets, it would be preferred if the aerosol and microbiology information are presented as columns for each animal rather than rows. This reviewer will defer to Clinical and Statistics for the appropriate format of telemetry and laboratory test results.

FDA Comment:

- **For the aerosol challenge sham dataset, you have provided the list of parameters and the formulas for some of the calculated parameters under the columns headings "Codes" and "Comments", respectively. However, there appears to be some typographical errors in the formulas used in the "Comments" column. For example, for the LD₅₀ equivalents in the "Codes column" the formula should be**

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**Calculated = INHDOSE/ [redacted] in the “Comments” column but is shown as
Calculated = AERO*TATV. Please check for accuracy of the formulas.**

Sponsor’s response:

The sponsor agreed that some of the formulas were incorrect. The data have been updated in the mock datasets and the data definitions file, accordingly.

FDA Comment#3:

It appears that different microbiological methods were used for collection, processing and culture at different laboratory sites for the efficacy and natural history studies. Such differences should be clearly documented in the study reports.

Sponsor’s response:

The sponsor agreed.

FDA Comment#4:

Data from any *in vitro* susceptibility studies conducted in different laboratories using a standardized method such as that recommended by the Clinical and Laboratory Standards Institute should be submitted for our review. We request the study reports should include the details of the microbiologic methods used as well as results of quality control strains included for testing.

Sponsor’s response:

The sponsor agreed.

CONCLUSIONS

The sponsor has responded to the Division request to provide “mock up” electronic datasets for the efficacy and natural history studies. The sponsor has agreed to the microbiology recommendations with the exception that further clarification was requested on the format of the datasets. The sponsor proposes to follow CDISC format in which each data point is represented by a row in the SAS dataset. It would be preferred if the aerosol and microbiology information are presented as columns for each animal rather than rows. However, this reviewer will defer to Clinical and Statistics for the appropriate format of telemetry and laboratory results.

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16 June 2011 SMS

Frederic J. Marsik, Ph.D., ABMM
Microbiology Team Leader DAIP
20 June 2011 FIN FJM

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

/s/

SIMONE M SHURLAND
06/21/2011

FREDERIC J MARSIK
06/21/2011