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RESEARCH**

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**STATISTICAL REVIEW(S)**



U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Drug Evaluation and Research  
Office of Translational Sciences  
Office of Biostatistics

## STATISTICAL REVIEW AND EVALUATION

### ANIMAL EFFICACY STUDY

**NDA/BLA Serial Number:** **NDA 20634/S-061**, SN 72, 76, 78, 80, 81, 85, 87, 88, 89; SDN 1244, 1248, 1250, 1253, 1255, 1259, 1262, 1263, 1264 (10/27/2011, 11/23/2011, 12/21/2011, 12/23/2011, 1/12/2012, 2/23/2012, 3/5/2012, 3/12/2012, 3/30/2012);  
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**Drug Name:** Levaquin<sup>®</sup> (levofloxacin) tablet

**Indication(s):** Pneumonic plague following exposure to *Yersinia pestis*

**Applicant:** Johnson & Johnson Pharmaceutical Research and Development L.L.C.

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## 1 EXECUTIVE SUMMARY

This supplementary NDA was submitted for the approval of levofloxacin (Levaquin<sup>®</sup>) for the treatment of pneumonic plague due to exposure to *Yersinia pestis* in adults and pediatric patients >50 kg and  $\geq$  6 months of age. Due to the rarity of this disease and ethical reason, efficacy studies can not be conducted in humans. Therefore, 21 CFR 314.610, “Approval based on evidence of effectiveness from studies in animals” (the Animal Rule), is considered an appropriate regulatory pathway for this application and that the single good laboratory practice (GLP) animal efficacy study, combined with known safety and efficacy profile of levofloxacin in other indications, information from the literature on rodents, along with clinical pharmacology data and natural history studies in African Green Monkeys (AGMs), is adequate for this application.

To meet the requirements of the Animal Rule, four natural history studies and one efficacy animal study in AGMs were included. The natural history studies demonstrated the lethality of a challenge dose which was at least 20 times the dose making 50% of AGMs dead (50% Lethal Dose or LD<sub>50</sub>s).

The efficacy study was a randomized, open-label, placebo-controlled study. The principle investigator making euthanasia decisions was blind to the assignment of treatment group. Sixteen animals in two cohorts were planned and enrolled. A protocol amendment decided to add 10 animals (8 on levofloxacin and 2 on placebo), approximately 7 months later after the completion of Cohort 2, in order to use a double-lumen catheter to draw blood for examining plasma levofloxacin concentration and to infuse levofloxacin or placebo. Therefore, 26 animals were randomized to the levofloxacin or placebo group (19 and 7 animals, respectively) and then 25 were challenged. Ten-day treatment (levofloxacin 8 mg/kg followed by 2 mg/kg administered approximately 12 hours later each day or placebo) was initiated within 6 hours after a body temperature elevated 1.5°C for at least 1 hour. The primary endpoint was survival up to Day 28 after challenge.

According to the sponsor’s analysis, the survival proportions were 94.1% (16/17) and 0% (0/7), for the levofloxacin group and placebo group, respectively, with a difference of 94.1% (exact 95% confidence interval: 55.5%, 99.9%) and a one-sided Fisher’s exact p-value <0.0001. We compared the survival proportions between the two treatment groups in ITT populations, assuming different scenarios for the two animals excluded from the sponsor’s primary analysis, in animals from Cohorts 1 and 2, animals with bacteremia at the initiation of treatment, animals with pulmonary infiltrates, and animals with a challenge dose greater than 20 LD<sub>50</sub>s. These survival analyses showed a consistent statistically significant difference between the two treatment groups.

The efficacy of levofloxacin in the treatment of pneumonic plague *due to Y. pestis* in AGMs was supported by this animal efficacy study. Whether or not the efficacy results can be a reliable indicator of its effectiveness in the treatment of human pneumonic plague depends upon the preclinical and clinical evaluation based on the Animal Rule.

Safety evaluation relies on the safety data from approved conditions for levofloxacin. There is no concern for safety.

## **2 INTRODUCTION**

The sponsor submitted this supplemental New Drug Application (sNDA) to support the use of Levaquin® (levofloxacin) in the treatment of pneumonic plague following the exposure to *Yersinia pestis* in adults and pediatric patients >50 kg and ≥ 6 months of age.

The supporting data are from the studies conducted by the National Institute of Allergy and Infectious Diseases (IND 64429). Four natural history studies and one animal efficacy study were conducted in Africa Green Monkeys (AGMs). FDA agreed on 2/7/2011 that 21 CFR 314.610, “Approval based on evidence of effectiveness from studies in animals” (the Animal Rule), is an appropriate regulatory pathway for this application and that the single good laboratory practice (GLP) animal efficacy study, combined with information from the literature on rodents, along with clinical pharmacology data and natural history studies in AGMs, is adequate for sNDA filing.

The efficacy animal study is selected for a full review. The four natural history studies will be reviewed briefly in the appendix of this document.

### **2.1 Overview**

*Y. pestis* is considered to be one of the most dangerous possible bio-weapons. There are no FDA–approved antibiotics for primary pneumonic plague due to the rarity of natural human disease and the paucity of antibiotic therapy studies in appropriate animal models.

Levofloxacin is a synthetic broad-spectrum antibacterial agent for oral and intravenous administration. It is currently approved and marketed in the United States [NDA 20-634 (Oral tablet), NDA 20-635 (Injectable), and NDA 21-721 (Oral solution)] to treat a number of infective conditions, including pneumonia and inhalation anthrax, post exposure.

Because of ethical concerns and the rarity of naturally occurring disease, evaluation of the efficacy of levofloxacin in the treatment of pneumonic plague relies on the results of animal models. Existing clinical and post-market safety data for currently approved indications are the basis for a safety assessment of levofloxacin for the treatment of pneumonic plague.

There is only one efficacy study included for this sNDA. Key information of this efficacy study is listed in the following table.

**Table 1: List of study included in analysis**

| Study    | Phase and Design                                | Treatment Period | Follow-up Period                             | # of Subjects per Arm             | Study Population                          |
|----------|---|------------------|--|-----------------------------------|---|
| FY-07-70 | Randomized<br>Placebo-controlled<br>Open-label* | 10 days          | 18 days (i.e.<br>28 days post-<br>challenge) | Control: 7<br>Levofloxacin:<br>19 | <i>Y. pestis</i><br>challenged<br>monkeys |

\*Survival up to day 28 was the primary efficacy endpoint. The principle investigator making euthanasia decisions was blinded to animal's treatment group.

In the general advice correspondence dated February 7, 2011, the Agency agreed that the data from the single GLP animal efficacy study conducted in AGMs combined with information from the literature on rodents, along with clinical pharmacology data on levofloxacin exposure and natural history studies in AGMs, are adequate to file an application for treatment of plague. The Agency considered the Animal Rule an appropriate regulatory pathway.

Four natural history studies, which are the basis for evaluation of the appropriateness of the proposed animal model and are shared with IND 64429 and Pre-IND 113289 application, will be reviewed briefly in the appendix.

## 2.2 Data Sources

Data sources include the study report and a SAS transport data set, fy07070, available in FDA data directory [\\edsub1\EVSPROD\NDA020634](#).

The study report contains appendices of the protocol and amendments, individual animal data, and the statistical analysis summary data.

*Comment: All protocol amendments were made after the initiation of the study; most of amendments were for clarification. Two amendments were about statistical methods for the primary and secondary analyses. Amendment 4 was about removal of one animal (X717) from the study for health reasons prior to aerosol challenge and amendment 6 was regarding the addition of Cohort 3. These issues will be addressed in later sections.*

Data set fy07070 is a SDTM like listing file, including some raw data and derived data such as hourly average telemetry data (temperature, heart rate, and respiratory rate). However, no analysis data sets or software analysis programs have been submitted. Some information, such as exposure time, challenge dose, and infusion time, etc was included in the study report, but was not available in the data set. There was no detailed information for animals removed from the study and for randomization in the initial submission. Therefore, additional data requests were sent to the sponsor to get essential data for evaluation. The requested data were submitted with good quality between November 2011 to March 2012 (SN76, 78, 80, 81, 85, and 87).

## 3 STATISTICAL EVALUATION

### 3.1 Data and Analysis Quality

The submitted data are of good quality. According to SN81, there are no case report forms available. All individual animal information was captured in each laboratory's data systems and was presented in the study report. Therefore, it is not possible to reproduce the primary analysis from case report forms. However, it is possible to reproduce the primary analysis from reported individual animal data, tabulation, and submitted data sets. It is possible to trace the survival endpoint from pathology data. Randomization codes were submitted on request and it is possible to verify treatment assignment.

### 3.2 Evaluation of Efficacy

#### 3.2.1 Study Design and Endpoints

This was a randomized, open-label, placebo-controlled animal efficacy study, conducted by Lovelace Biomedical and Environmental Research Institute for the National Institute of Allergy and Infectious Disease (NIAID) between 2/15/2008 and 2/20/2009. The objective of this efficacy study was to evaluate the efficacy of intravenous levofloxacin in preventing death due to inhaled *Y. pestis* at a target dose of 100 LD<sub>50</sub>s in AGMs.

AGMs, with implanted telemeters, intravenous catheters in the femoral vein for blood withdrawal and infusion of medication, were assigned to the study. The animals were approximately 3 - 8 kg and at least 2 years old when assigned to the study (animals were wild-caught. Therefore, ages were actually unknown).

Eight (8) females and 8 males were planned to be enrolled. In each of the two cohorts of 8 AGMs, 5 to 6 AGMs would receive levofloxacin and 2 to 3 would receive placebo for a total of 5 control animals. Animals were randomized into the treatment and control groups prior to aerosol exposure. All animals were planned to be exposed to target dose of 100 LD<sub>50</sub>s on Day 0. Established pneumonic plague was indicated by a body temperature of greater than 39°C for at least one hour, which was the signal to initiate infusions. Animals were planned to be treated within six hours of onset of fever with either the test article, levofloxacin (5 mg levofloxacin/mL 5% dextrose), or control article, 5% dextrose. Test or control article was administered twice daily to match the pharmacokinetics of the antibiotic in humans, due to a more rapid clearance of levofloxacin in AGMs (levofloxacin 8 mg/kg followed by 2 mg/kg administered approximately 12 hours later).

*Comments: According to the protocol and study report, animals would be assorted into test groups using a validated computerized data acquisition system based on body weights. Animal was randomized into housing placement and exposure order using Microsoft Excel random number generator. This randomization had nothing to do with the randomization of animals to treatment. Randomization to the two treatment groups was based on separate randomization codes.*

*Temperature would be recorded by software every five minutes and averaged for hourly temperature at the end of the study. Therefore, in this review, onset of fever and resolution of fever were based on hourly data, which might be slightly different from values derived from the temperature data recorded every five minutes.*

Infusions of levofloxacin or control were continued until death, moribund euthanasia or 20 infusions had been completed. Recorded telemetry data were to be inspected twice daily during the study and every 4-6 hours during the clinically critical phase of the study [i.e, onset of infection and evidence of morbidity/moribundity (e.g. Days 2-7)].

The primary efficacy was survival (at Day 28). This was an open-label study. However, the principle investigator making euthanasia decisions for moribund animals was blind to the assignment of treatment group.

*Comment: We recommended the study be blinded. However, it was not blinded. Here is the communication regarding blinding of this study:*

- ***FDA's comment sent on 12/10/07 on study protocol (SN34, submitted on 10/3/07):** We suggest that the study be blinded.*
- ***Sponsor's response in SN 36 (SDN 40, 2/08/2008):** Because of the logistics of working in a BSL3, Selected Agent laboratory, blinding the study is not feasible.*
- ***FDA general comment on SN36 sent on 2/29/2008:** Blinding this study is important if this study will be used at a pivotal efficacy study. It is unclear why blinding the study to treatment is not feasible and other similar studies were blinded.*
- *On 4/29/2010 (SN43, SDN47), a final study report was submitted.*

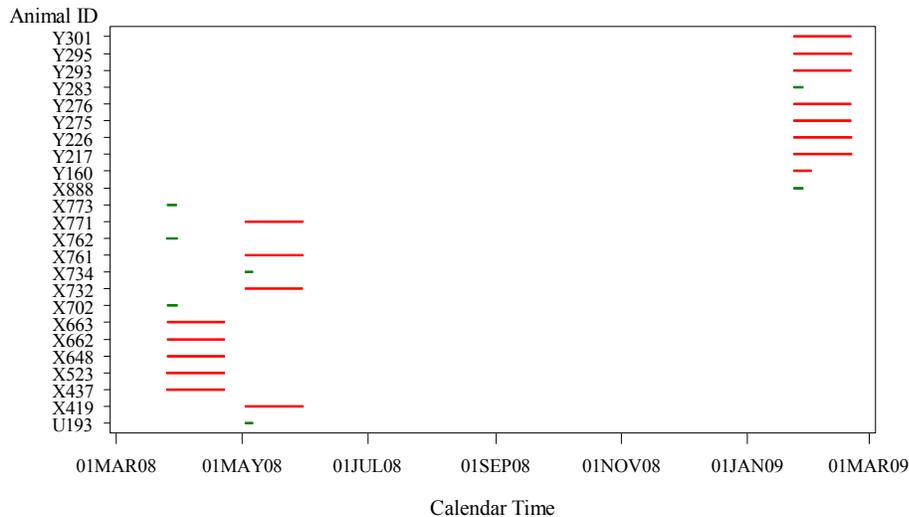
There was no justification in the protocol for the sample size used in the study. We requested this information when the protocol was initially reviewed, but did not receive a response.

The first two cohorts were enrolled as planned. However, approximately 7 months after completing Cohort 2, Cohort 3 containing 10 animals (2 on control and 8 on levofloxacin) was added (see Figure 1) in protocol amendment #6 (effective 12/22/2008). The amendment states "For Cohort 3, dual-lumen/port Hickman catheters will be used. An additional Cohort was added to the study". After requesting for further explanation for addition of this cohort, we received the following response dated 2/23/2008 (SN85): Cohort 3 was added in order to use a double-lumen catheter to draw blood and infuse treatment in order to test plasma levofloxacin concentration.

*Comment: This explanation sounds reasonable. However, as this was an open-label study with a small sample size. Given the timing of conducting the three cohorts (see Figure 1), it is likely that the data from the first two cohorts were analyzed prior to conducting the third cohort and the results could have affected the decision to add cohort 3. We will conduct a sensitivity analysis for the primary efficacy endpoint without using the data from Cohort 3 in a following section.*

*Comment: Randomization ratio was not 1:1, but approximately 3:1 to 4:1. In SN 85 (2/23/2008), the sponsor provided an explanation for using 4:1 in Cohort 3 as follows: “This ratio was based on historical experience across several labs where natural history and control animals routinely succumb to disease and practical limitations on the number of animals that can be handled according to the protocol at one time”.*

**Figure 1. Time lines from challenge to death or euthanasia**



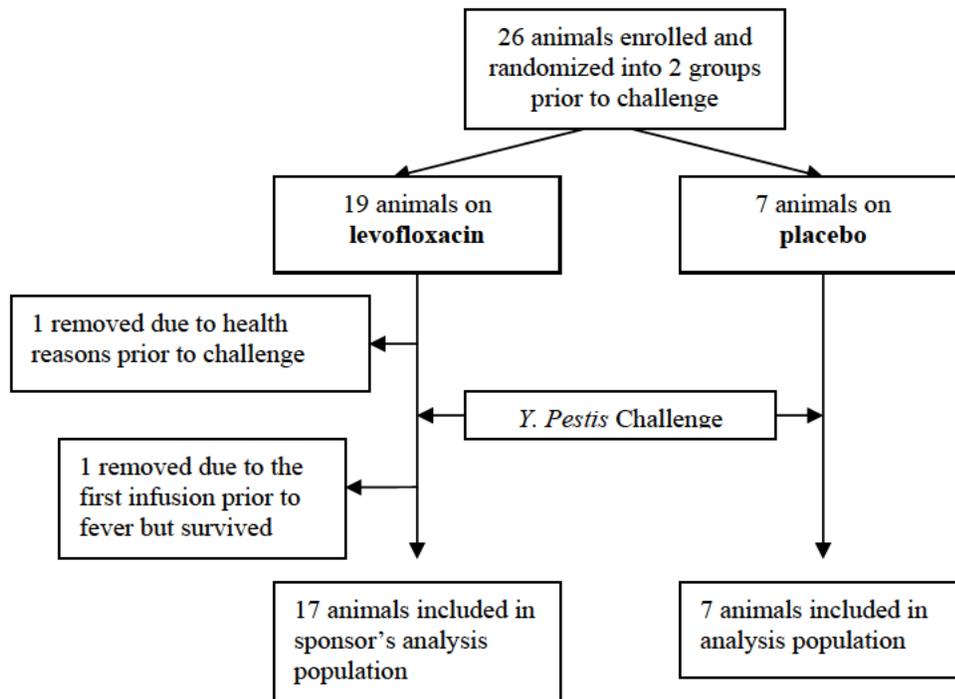
### 3.2.2 Patient Disposition, Demographic and Baseline Characteristics

AGMs were enrolled into the study and challenged to 3-145 LD<sub>50</sub>s to *Y. pestis* (target dose: 100 ± 50 LD<sub>50</sub>s, or approximately [redacted] colony forming unit (CFU)/ml) on Day 0. Following challenge, the animals were monitored via telemetry to determine the onset of defined fever (a mean fever greater than 39°C for more than one hour recorded by telemetry).

The following flowchart shows the disposition of the study. Two animals were removed from Cohort 2 after randomization: animal X717 prior to challenge due to health reasons and animal X779 infused with test article prior to the onset of fever. According to a pre-study case report form, animal X717 was not a good candidate for the study due to concerns over past TB suspect results and lung disease. Animal X779 also received 0.5 mL of a 6.29 mL dose of 5 mg/kg levofloxacin on Day 9, and survived to Day 28 with no additional treatment.

*Comment: It appears reasonable to remove animal X717 from all analyses. It is less clear how animal X779 should be handled. Sensitivity analyses will be conducted with and without these animals and with different assumed endpoints.*

**Figure 2: Animal disposition and analysis population**



Eighteen (10 females and 8 males) were treated with levofloxacin and 7 (3 females and 4 males) received placebo control.

Animals were planned to receive 20 infusions. Animal Y295 received 6.5 mg of levofloxacin at the 12<sup>th</sup> infusion when it was supposed to receive 5 mg. This protocol deviation should not have much effect on the outcome of this animal.

The following table shows the demographic and baseline information by treatment group for the animals included in the sponsor's primary efficacy analysis. The animals in the control group were 0.49 kg heavier, on average, in body weight than animals in the levofloxacin group.

From the following table and figure, it seems that the challenge doses were smaller in the levofloxacin group than in the control groups by study cohort and that Cohort 2 received much higher doses than Cohort 1. Cohort 3 also received much lower doses than the previous 2 cohorts. In the study report (page 284) the sponsor states that that challenge doses for the first 4 animals in Cohort 3 were lower than cohorts 1 and 2 because the bacteria growth curve may have overestimated the actual concentration for Cohort 3. The doses for the last 6 animals in Cohort 3 (Y160, Y226, Y275, Y276, Y295, and Y301, all in the levofloxacin group) were much lower than expected and than the first 4 animals in the same cohort, presumably due to an additional reason. The sponsor states in the study report (page 284) that it may have been the result of technician dilution error.

**Table 2: Demographic and baseline characteristics and *Y. pestis* challenge dose by treatment**

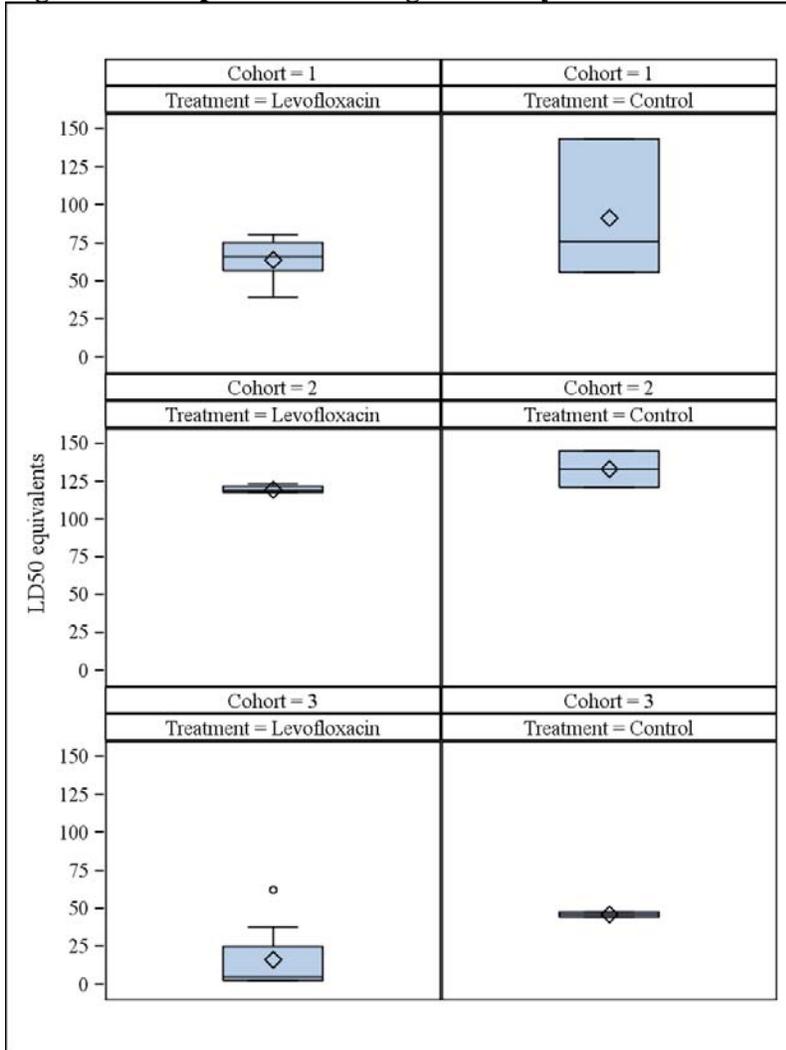
|   | Levofloxacin<br>N=17 | Control<br>N=7 |
|---|----------------------|----------------|
| Number of animals by cohort   |                      |                |
| 1   | 5                    | 3              |
| 2   | 4                    | 2              |
| 3   | 8                    | 2              |
| Gender n(%)   |                      |                |
| Male  | 8 (47%)              | 4 (56%)        |
| Body weight at pre-study (kg)<br>Mean (SD)  |                      |                |
| Cohort 1  | 4.59 (0.88)          | 5.62 (1.03)    |
| Cohort 2  | 4.87 (0.92)          | 4.99 (0.88)    |
| Cohort 3  | 5.01 (1.22)          | 5.26 (1.92)    |
| All animals   | 4.85 (1.02)          | 5.34 (1.09)    |
| <i>Y. pestis</i> CO92 aerosol<br>challenge dose (LD <sub>50</sub> s)<br>Mean (SD) n |                      |                |
| Cohort 1  | 63.7 (16.2)          | 91.6 (46.0)    |
| Cohort 2  | 119.9 (2.6)          | 133.3 (17.0)   |
| Cohort 3  | 16.2 (22.0)          | 45.9 (2.2)     |
| All animals   | 54.59 (45.9)         | 90.4 (45.0)    |

SD: Standard deviation. Body weight for X779 (female, received a challenge dose of 83 LD<sub>50</sub>s) was 4.44 kg; for X717 (male) 4.31 kg. These two animals were not included in this table.

*Comment: According to the Wilcoxon Two-Sample Rank Sum Test (exact), the one-sided p-value for the comparison of challenge dose between treatment arms was 0.0497 and two-sided p-value was 0.0995. This difference appears primarily due to the 6 animals randomized to levofloxacin in cohort 3 who received very low challenge doses. Since all animals in Cohorts 1 and 2 and control animals in Cohort 3 received at least 44 LD<sub>50</sub>s, the lethality of the received doses for these animals can be supported with the study results from the natural history studies (at levels of 20 LD<sub>50</sub>s or higher, all animals died). See Appendix for more details. Therefore, the difference in doses seen in animals other than the levofloxacin treated animals in cohort 3 should not be a concern.*

*It is important to note that unlike the animals with low LD<sub>50</sub>s in the natural history studies, these 6 levofloxacin animals had positive blood culture results and had onset of fever, indicating that the animals had infections. Additionally, though their LD<sub>50</sub> values are reported to be low, all of these LD<sub>50</sub> values are based on estimation and all have very wide confidence intervals. However, it is not possible to conclude that the doses were lethal. Sensitivity analyses will be conducted to address this issue.*

**Figure 3: Box plots of challenge doses by cohort**



X779's challenge dose of 83 LD<sub>50</sub>s was not included in the levofloxacin Cohort 2.

### 3.2.3 Statistical Methodologies

#### Primary Endpoint

The primary endpoint was survival at 28 days after challenge.

*Comment:* [REDACTED] (b) (4)  
 After receiving FDA's comments, the primary endpoint was changed to survival. The following are the comments that were sent to the sponsor:

*Comment on SN034 (12/10/07):* The study protocol [REDACTED] (b) (4)  
 [REDACTED] Given the influence of euthanasia criteria, inherent inter-animal variation, and the required relationship between the endpoints in the animal

*study and those desired in humans, time to death should be considered a secondary endpoint, (b) (4)*

*SN36/SDN40 Sponsor's response (2/8/2008): The primary endpoint is survival. Time to death will be used as a secondary endpoint. This has been changed in the protocol.*

## **Secondary Endpoints**

Secondary endpoints included the following:

- Time to death
- Serum chemistries (baseline, Days 2, 6, and 28)
- Hematology (baseline, Day 2, 6, and 28)
- Body Weight (baseline, Day 0, and day of necropsy or euthanasia)
- Telemetry (hourly averages at baseline and each study day)
- Levofloxacin levels (baseline, study days 1, 3 (pre- and pos-infusion), 4, 6 (pre- and pos-infusion), and 19 (pre- and pos-infusion)).
- Microbiology data (baseline, study days 2,3,4,5,6,7, 14, 21, and euthanasia)

## **Analysis population**

There were no analysis populations defined in either the protocol or study report. In that case, it would generally be assumed that the intent-to-treat population would be used for the primary analysis. The intent to treat population is typically defined as all randomized subjects. However, the sponsor excluded two animals from the primary analysis. The two animals which have been described previously were not included in the sponsor's analysis: X717 which was eliminated prior to challenge due to health reasons and X779 which received the test article before the onset of fever.

*Comment: The review team has determined that exclusion of animal X717 is appropriate given that this animal was not challenged. However, it is not as clear how animal X779 should be handled in the analysis. As this animal survived, exclusion of this animal from the survival analysis is more conservative than including this animal as a survival since this animal was randomized to levofloxacin. Because this is a conservative analysis, we believe it is acceptable to exclude this animal. See the efficacy section below for sensitivity analyses.*

## **Analysis methods for the primary endpoint**

The survival rates in the two treatment groups will be compared using Fisher's exact test.

*Comment: Since Fisher's exact test does not have the property that the two-sided p-value is twice that of the one-sided p-value, a one-sided p-value from a Fisher's exact test with a cut-point of 0.025 should be used for efficacy evaluation.*

## Analysis methods for secondary endpoints

The original protocol did not specify analysis statistical methods for the secondary endpoints. In amendment #2, methods for secondary endpoints were added for clarification as follows:

All secondary endpoints would be considered exploratory. Analyses of secondary endpoints may be performed if significance was found with the primary analysis. The analyses would be adjusted for type I error, by ordering the secondary analyses, by a Bonferroni adjustment for the secondary analyses or as deems appropriate by the statistician.

*Comment: The methods for adjustment of type I error were not clear. Actually the conducted analyses for the secondary analyses were descriptive in the study report and did not adjust for the type I error. But as they were considered purely exploratory, this is acceptable.*

In protocol amendment #11, more detailed analyses for secondary endpoints were included as an addendum, not as a replacement to the existing methods, as follows:

Statistical analysis of some secondary endpoints (such as serum chemistries, hematology and telemetry) was performed using a repeated measures analysis of variance (RMANOVA). The values would be transformed to differences from the appropriate baseline values. The baseline measurements should be the average of any measurements taken 1 to 2 week prior to challenge. For the telemetry data, the baseline would be the average of the times prior to treatment matched to the appropriate times post-treatment. Variables to be included in the model were also specified.

Quantitative bacteriology values were transformed by taking Log<sub>10</sub> (CFU value +1), since there were 0 values.

Due to the deaths of all animals in the control group, actual statistical analyses that involved comparisons of the two groups were limited to data collected through study day 6 for serum chemistry and hematology and study day 4 for quantitative bacteriology and telemetry.

*Comments: Due to the vagueness of the analyses of the secondary endpoints as stated in the protocol or amendments, we consider them as purely exploratory, as did the sponsor. We only include temperature and Log<sub>10</sub> (CFU+1) in blood in the efficacy section in this review. Other secondary endpoints are not included.*

### 3.2.4 Results and Conclusions

#### Onset of fever

A summary of time from challenge to onset of fever (becoming febrile) by treatment group is shown in the following table (animal X779 was excluded):

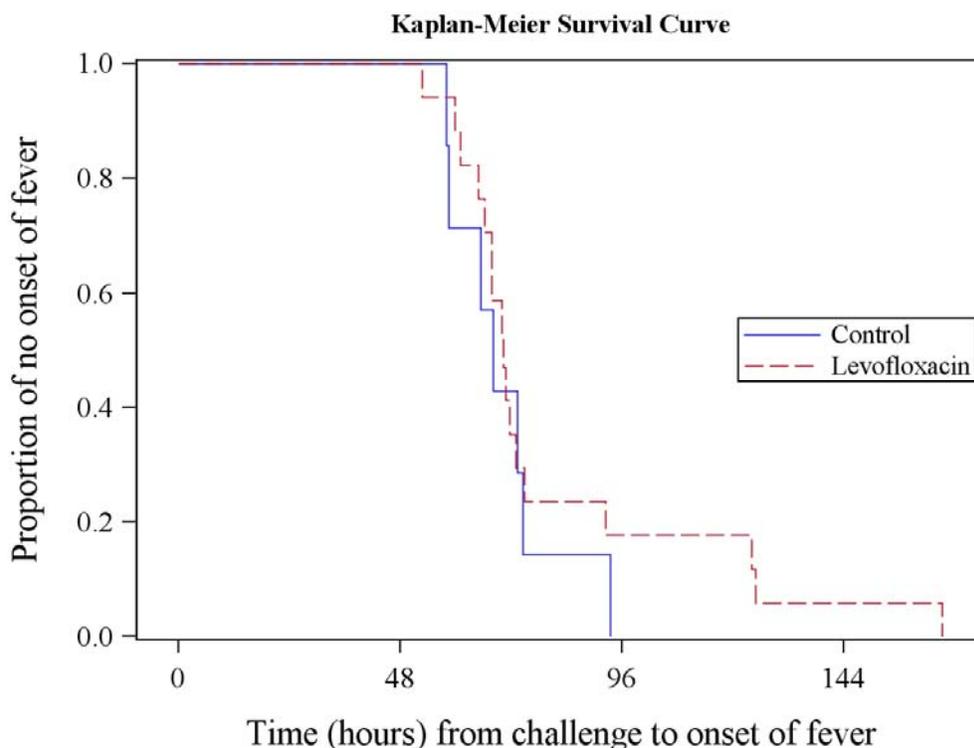
**Table 3: Time from challenge to onset of fever and time from onset of fever to 1<sup>st</sup> infusion**

| Time to onset of fever (hrs) | Levofloxacin<br>N=17 | Control<br>N=7 |
|------------------------------|----------------------|----------------|
| Mean                         | 81.09                | 70.23          |
| SD                           | 29.60                | 12.10          |
| Median                       | 70.40                | 68.23          |
| Range                        | 52.82, 165.35        | 58.02, 93.43   |

Adapted from Table 11 in the Study Report with medians and ranges added from reviewer's analyses.

All 17 animals in the levofloxacin group had onset of fever within 168 hours (or 7 days) after the start of challenge. The excluded animal X779 also developed fever within 168 hours, at 90 hours, post challenge. All animals in the control group had onset of fever within 96 hours (4 days). Three animals in the levofloxacin group had onset of fever after 96 hours of challenge, which caused this group to have a longer time to fever, although the difference was not statistically significant.

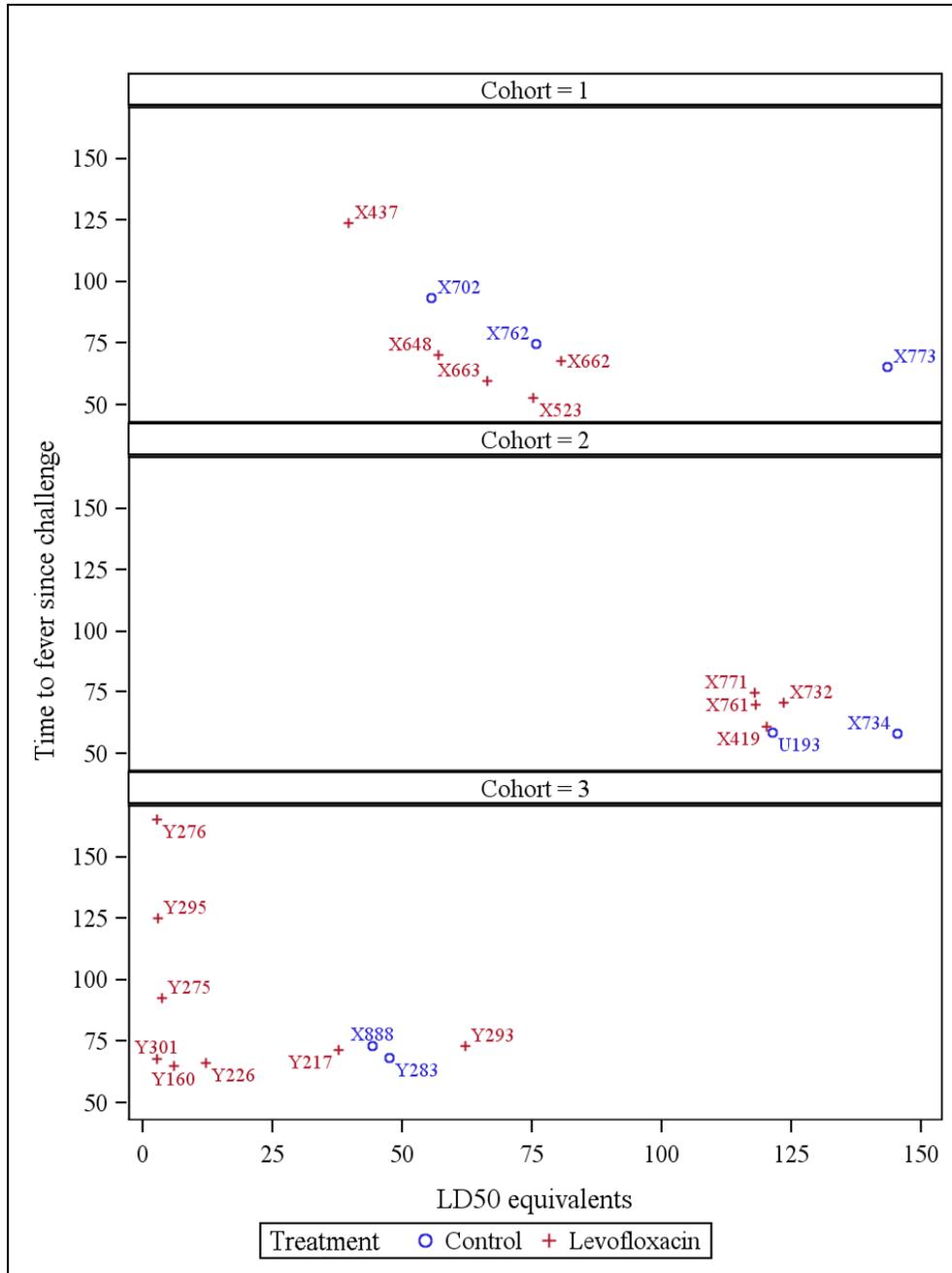
**Figure 4: Time from challenge to onset of fever in hours by treatment group**



The following figure, Figure 5, contains three graphs, one for each cohort, of time from challenge to the onset of fever by LD<sub>50</sub> challenge dose. As shown in Figure 5, among the 5 animals in Cohort 3 in the levofloxacin group with a challenge dose less than or equal to 6.04 LD<sub>50</sub>s, 3 animals had an onset of fever later than 92 hours. Note that the animal with 6.04 LD<sub>50</sub>s, Y160, was the only animal which did not survive in the levofloxacin group. See efficacy analysis section for more information of this animal.

*Comment: Out of the four natural history studies, only two animals survived. These two animals were from the USAMRIID study (F03-09G). These two animals were exposed to 9 and 12 LD<sub>50</sub>s. All animals which were challenged with a dose greater than 21 LD<sub>50</sub>s died. Because of this finding, the lethality of challenge of less than 20 LD<sub>50</sub>s is questionable. See the appendix for more information on the natural history studies. Therefore, it is reasonable to eliminate these animals with a challenge dose less than 20 LD<sub>50</sub>s. See sensitivity analysis below.*

**Figure 5: Time from challenge to onset of fever in hours by cohort and treatment group**



### Time from Onset of Fever to Initiation of Treatment

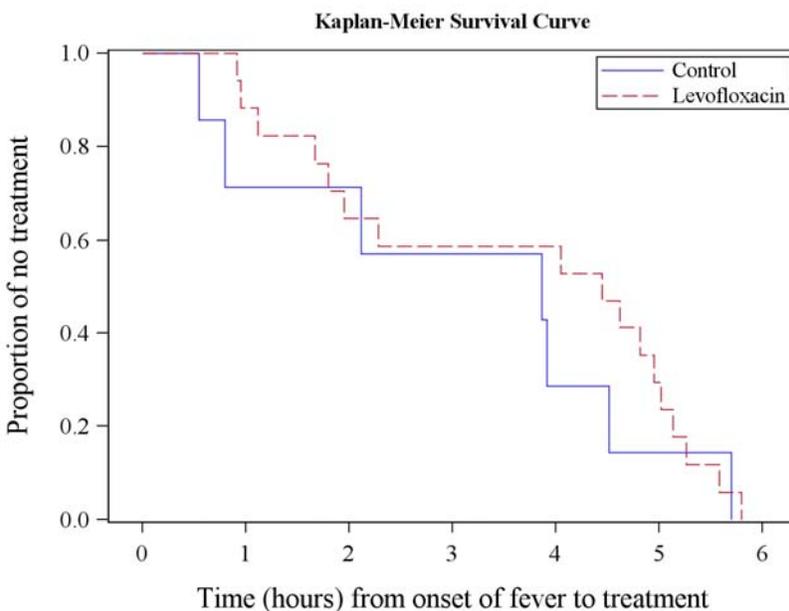
As the following table and figure show, all animals, excluding animal X779, received the first infusion within 6 hours after onset of fever, with a mean of 3.55 and 3.07 hours for the levofloxacin and control groups, respectively. It appears that there was no difference in time to receipt of the first dose of treatment.

**Table 4: Time from onset of fever to 1<sup>st</sup> infusion**

| Time from onset of fever to initiation of treatment (hrs) | Levofloxacin<br>N=17 | Control<br>N=7 |
|---|----------------------|----------------|
| <b>All cohort</b>   |                      |                |
| Mean  | 3.55                 | 3.07           |
| SD  | 1.82                 | 1.95           |
| Median  | 4.45                 | 3.87           |
| Range   | 0.92, 5.80           | 0.55, 5.70     |

Adapted from Table 11 in the Study Report with medians and ranges added from reviewer's analyses.

**Figure 6: Time from onset of fever to treatment (1<sup>st</sup> infusion) in hours by treatment group**



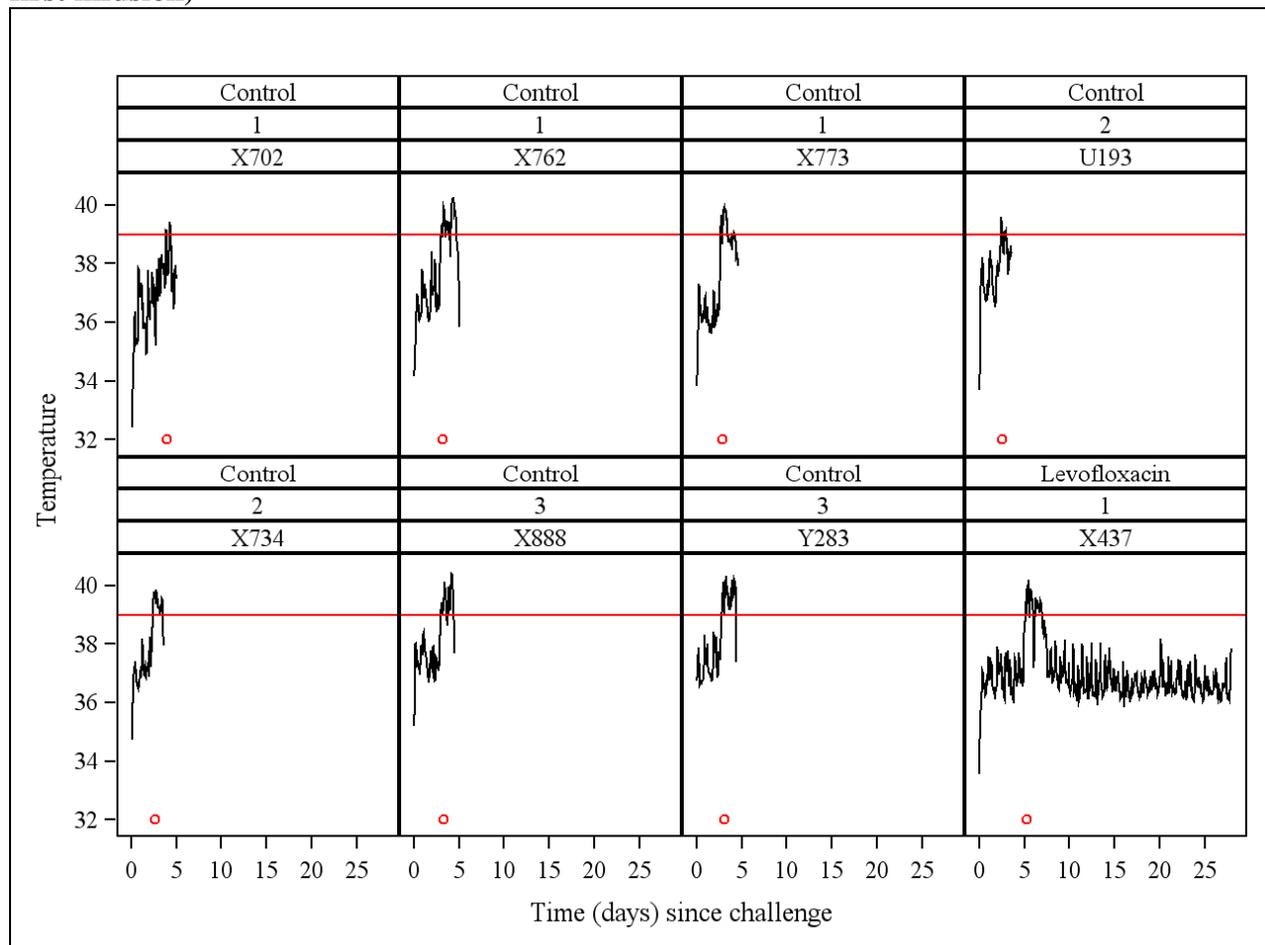
### Time from First Treatment to High Fever Resolution

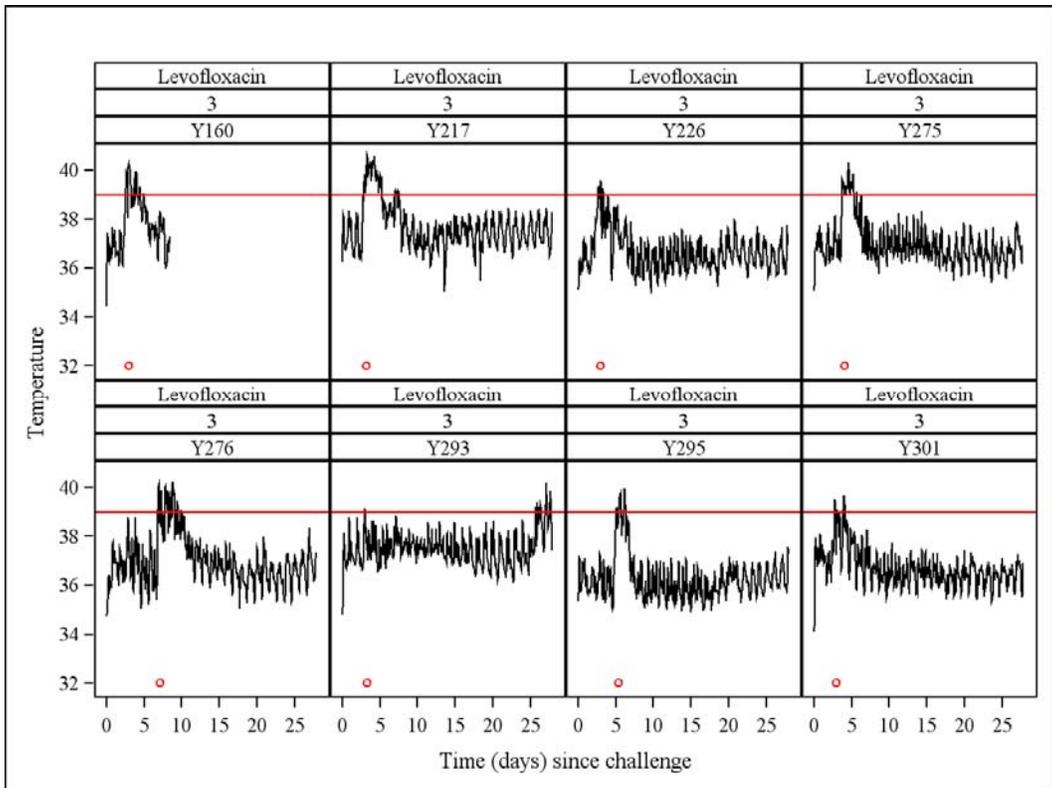
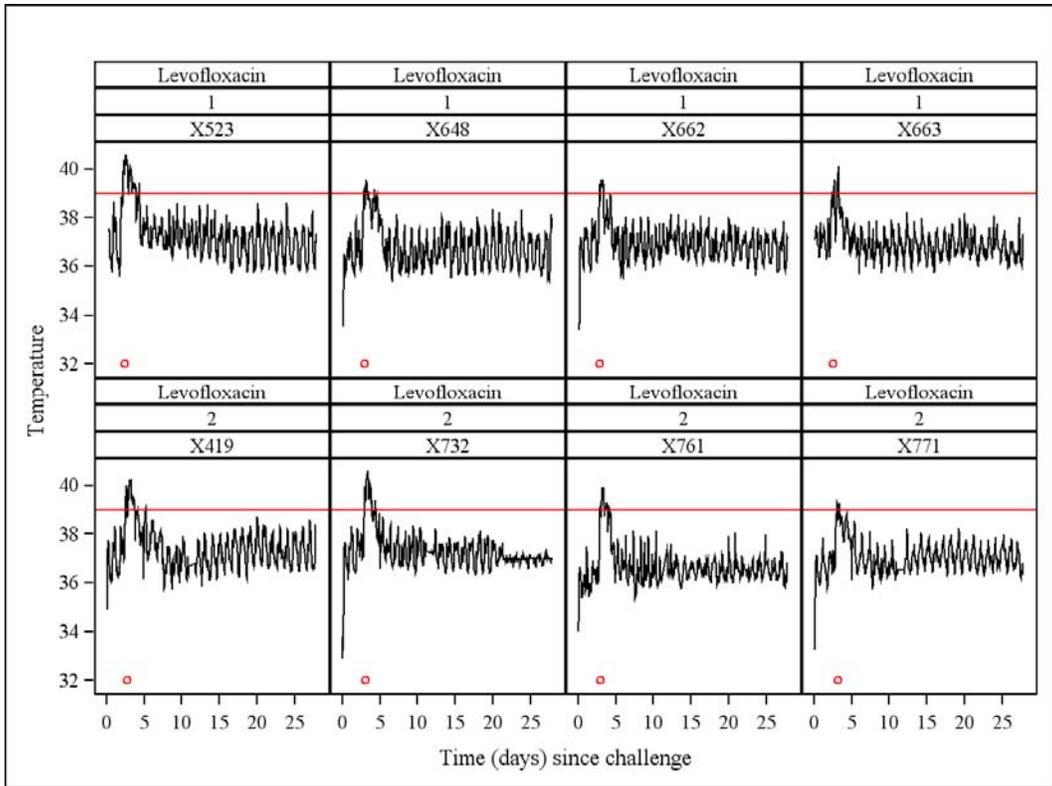
The temperature over time since challenge is shown in the following figure. The red circle indicates the time of first infusion. All animals had onset of fever after challenge as discussed before. In the levofloxacin group, all animals, except animal Y293, had their fever drop to below 39°C in 6.2 to 104.4 hours after initiation of the treatment (38.6 hours on average). For the

control group, high fever resolved in 25.0 hours on average with a range of 10.6 to 38.0 hours after initiation of first infusion, and very soon before death.

It is noted that animal Y293 had a fever in the last 3 days. According to SN 86, “upon termination of the study,  $>10^3$  CFU/s/mL of a non-*Y. pestis* bacterial contaminant(s) were isolated from the vascular catheter”. “Blood samples lacked detectable CFUs, suggesting that the non-*Y. pestis* bacteria was localized to the catheter”. “Although a definitive explanation for the fever can not be provided, the bacterial growth linked to this catheter may have contributed to the elevated temperature of Y293”. This increase in temperature was not considered a problem for the primary endpoint analysis by the medical reviewer.

**Figure 7: Temperature since challenge for each animal by treatment and cohort (red circle, first infusion)**





## Log (CFU+1) over Time

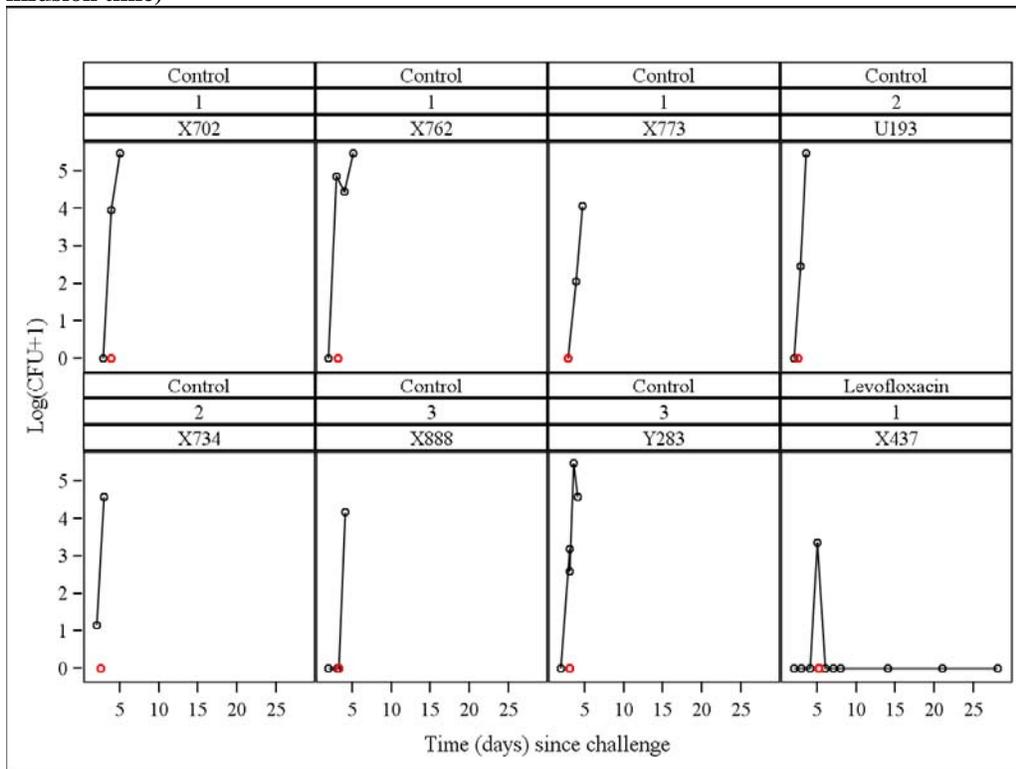
The following figures show the log (CFU/ml+1) in blood over time and the first infusion time (red circle). Most of animals received the first infusion at or before the peaked CFU level in blood samples collected daily.

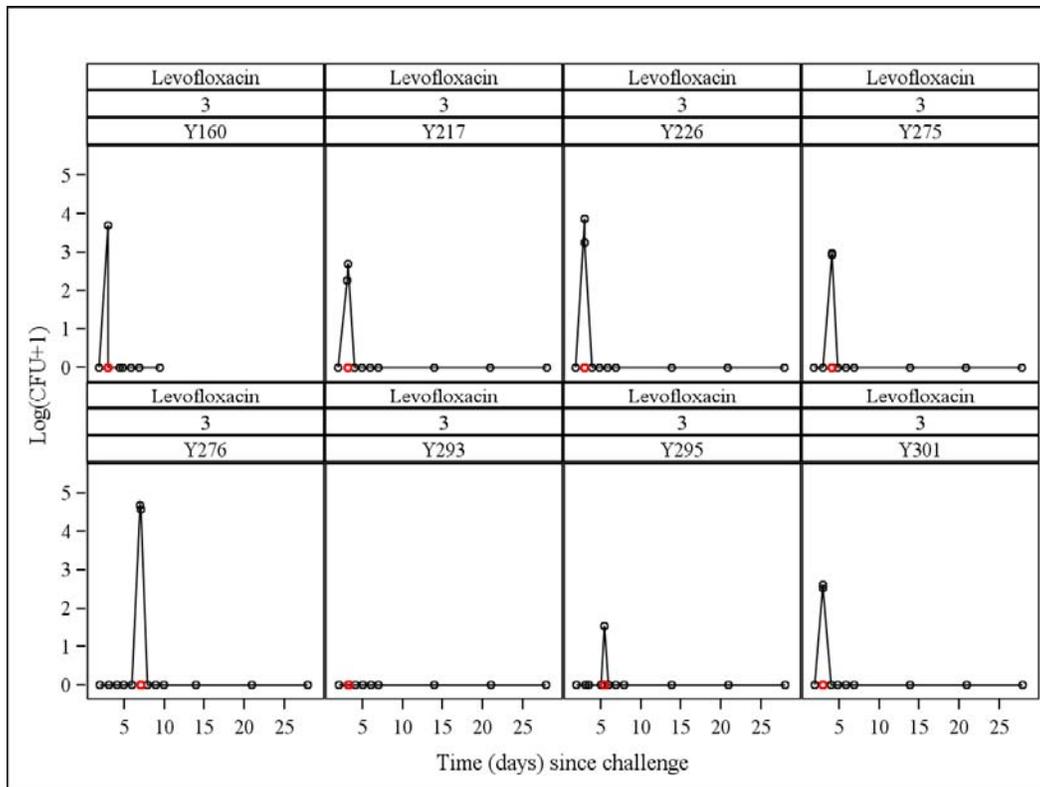
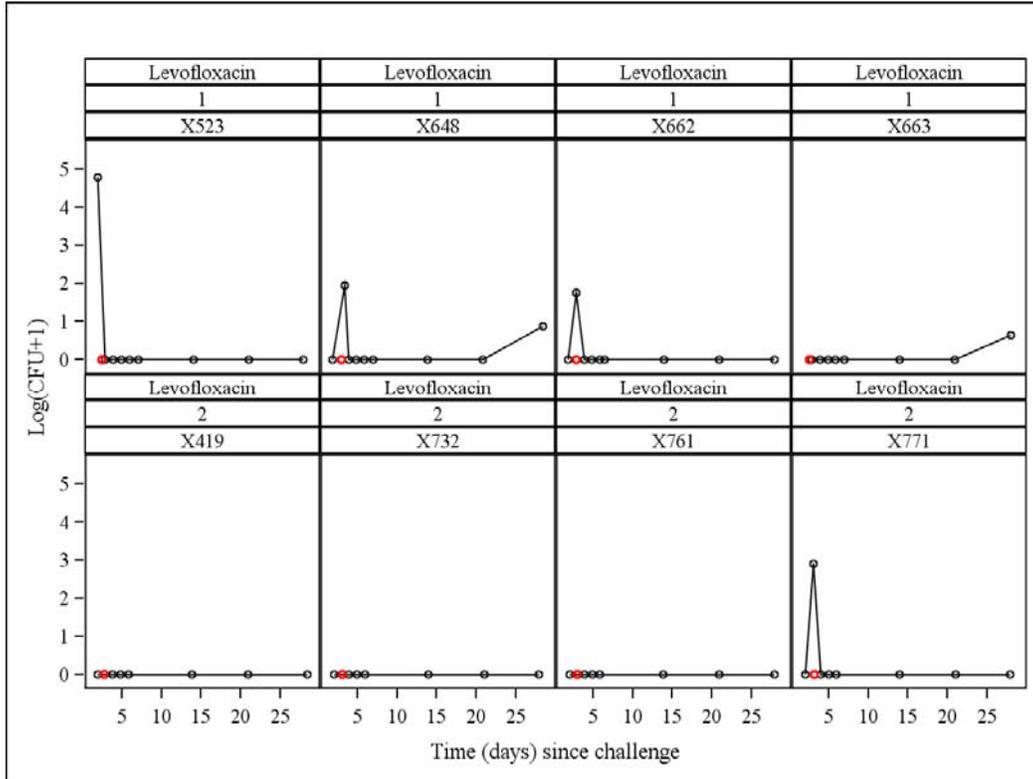
Two animals (X648 and X663) in the levofloxacin group in Cohort 1 had positive *Y. pestis* blood culture at terminal visit (log (CFU/ml+1)=0.88 and 0.64 (or CFU/ml 6.7 and 3.3), at hours 682 and 671 after start of challenge, respectively). These animals were survivors and are considered as such for the primary endpoint of survival. However, the clinical and microbiological reviewers considered these two animals and an animal (X523) with a positive tissue culture result as microbiological failures in an additional analysis.

Five animals (X419, X663, X732, X761, and Y293) in the levofloxacin group (first 4 animals from Cohorts 1 and 2 and the last one from Cohort 3) had no positive blood culture results throughout the entire study period. However, the 4 animals in Cohorts 1 and 2 had pulmonary infiltrates which is important given the indication is “pneumonic plague”, regardless of blood culture results. The animal in cohort 3, Y293, with a challenge of 62 LD<sub>50</sub>s had baseline chest radiographs only and no chest radiograph was available after challenge.

*Comment: Additional sensitivity analyses will be based on both positive blood culture as well as chest radiographs in the section below.*

**Figure 8: Log (CFU+1) in blood over time for each animal by treatment group and cohort (red circle: first infusion time)**





## Efficacy results

The following table shows, for each animal cohort, the challenge dose, bacteremia at initiation of treatment, chest radiograph (pulmonary infiltrates) at Day 5, survival, and culture result at the terminal visit for the animals. Based on these variables, different sensitivity or subgroup analyses for the primary endpoint will be conducted.

**Table 5: Treatment and selected outcomes for each animal**

| Animal | Cohort | Treatment | Sex | Aerosol Challenge (LD <sub>50</sub> ) | Bacteremia | Pulmonary Infiltrates | Survival to Day 28 | Y. Pestis Culture at Terminal Visit |
|--------|--------|-----------|-----|---------------------------------------|------------|-----------------------|--------------------|-------------------------------------|
| X702   | 1      | Control   | F   | 56                                    | +          |                       | No                 | -                                   |
| X773   | 1      | Control   | M   | 143                                   | -†         | +                     | No                 | -                                   |
| X762   | 1      | Control   | M   | 76                                    | +          | +                     | No                 | -                                   |
| X734   | 2      | Control   | M   | 145                                   | +          |                       | No                 | -                                   |
| U193   | 2      | Control   | F   | 121                                   | +          | +                     | No                 | -                                   |
| X888   | 3      | Control   | F   | 44                                    | -†         |                       | No                 | -                                   |
| Y283   | 3      | Control   | M   | 47                                    | +          |                       | No                 | -                                   |
| X762   | 1      | Levo      | M   | 76                                    | +          | +                     | Yes                | -                                   |
| X437   | 1      | Levo      | M   | 40                                    | +          | +                     | Yes                | -                                   |
| X662   | 1      | Levo      | F   | 81                                    | +          | +                     | Yes                | -                                   |
| X663   | 1      | Levo      | F   | 66                                    | -          | +                     | Yes                | +                                   |
| X648   | 1      | Levo      | F   | 57                                    | +          | +                     | Yes                | +                                   |
| X717   | 2      | Levo      | M   | Not challenged                        |            |                       | Removed            |                                     |
| X419   | 2      | Levo      | F   | 120                                   | -          | +                     | Yes                | -                                   |
| X732   | 2      | Levo      | F   | 124                                   | -          | +                     | Yes                | -                                   |
| X761   | 2      | Levo      | M   | 118                                   | -          | +                     | Yes                | -                                   |
| X771   | 2      | Levo      | M   | 118                                   | +          | +                     | Yes                | -                                   |
| X779*  | 2      | Levo      | F   | 83                                    | -          | Not known             | Yes                | -                                   |
| Y160   | 3      | Levo      | F   | 6                                     | +          |                       | No                 | -                                   |
| Y217   | 3      | Levo      | F   | 38                                    | +          |                       | Yes                | -                                   |
| Y226   | 3      | Levo      | F   | 12                                    | +          |                       | Yes                | -                                   |
| Y275   | 3      | Levo      | M   | 4                                     | +          |                       | Yes                | -                                   |
| Y276   | 3      | Levo      | M   | 3                                     | +          |                       | Yes                | -                                   |
| Y293   | 3      | Levo      | M   | 62                                    | -          |                       | Yes                | -                                   |
| Y295   | 3      | Levo      | F   | 3                                     | +          |                       | Yes                | -                                   |
| Y301   | 3      | Levo      | M   | 3                                     | +          |                       | Yes                | -                                   |

\*Removed from study due to initiation of treatment prior to fever.

†Became bacteremic the day after the first infusion.

***Sponsor's primary analysis from the study report***

The survival rates at Day 28 are reported in the following table. There was a significant difference in survival proportions between the levofloxacin (94.1%) and the control group (0%). The reported Fisher's exact p-value was <0.001.

**Table 6: Survival (%) for all animals and by study cohort**

|          | Levofloxacin<br>N=17 | Control<br>N=7 |
|----------|----------------------|----------------|
| Total*   | 16 (94.1%)           | 0              |
| Cohort 1 | 5/5 (100%)           | 0/3            |
| Cohort 2 | 4/4 (100%)           | 0/2            |
| Cohort 3 | 7/8 (87.5%)          | 0/2            |

\* Fisher's exact p-value (one-sided):  $2.31E-5 < 0.0001$ . Exact 95% confidence interval for the difference in survival proportions: 55.5%-99.9%.

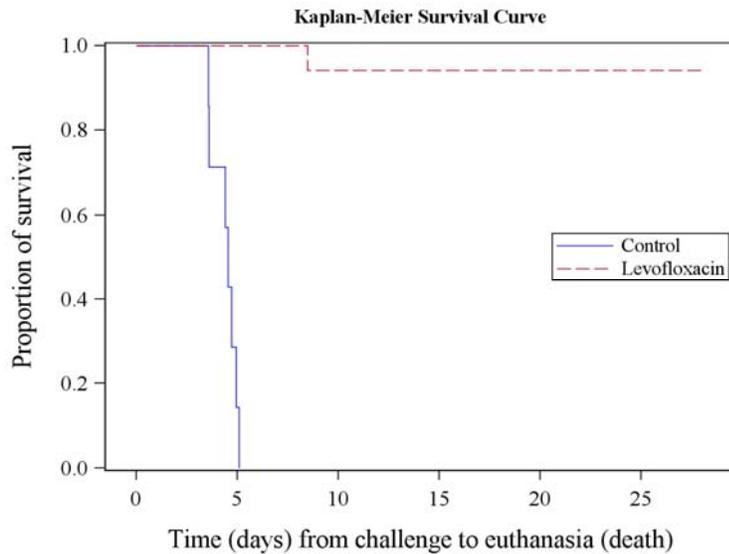
Animal Y160 in Cohort 3, with a challenge dose of 6.04 LD<sub>50s</sub>, was euthanized at 203 hours (on Day 8) after challenge and was considered as treatment failure in the sponsor's primary efficacy analysis. Blood culture was positive only immediately prior to the initiation of treatment on Day 3. All daily blood cultures (Days 2-7) and tissues collected at necropsy were negative. Necrosis of the gastric epithelium was found from histopathological examination. There was no apparent evidence of *Y. pestis* infection in other organs.

*Comment: During the Advisory Committee meeting held on April 4, 2012, the sponsor presented as their primary ITT analysis, an analysis that included animal X779 as a treatment failure, despite the fact that this animal survived. Their survival result for Levofloxacin was 16/18 and for placebo was 0/7 with a p-value for the difference of < 0.001.*

***Sponsor's survival analysis from the study report including 3 cohorts***

The survival curves of all animals using the Kaplan-Meier method from the two treatment groups are shown in the following graph. Animals in the levofloxacin group had a higher proportion of survival than animals in the control group.

**Figure 9: Time from challenge to euthanasia (death) by treatment group**



***FDA’s ITT analysis for all randomized animals***

In this analysis, X717 (removed, but considered as a survivor) and X779 (survived) were included as survivor because they were randomized. The following table shows the efficacy results from this ITT population.

**Table 7: Survival at Day 28 post exposure for all randomized animals (both X717 and X779 as survivors), ITT population**

|              | <b>Levofloxacin</b> | <b>Placebo</b> |
|--------------|---------------------|----------------|
| Survival (%) | 18/19 (94.7%)       | 0/7            |
| 95% CI       | 56.6%, 99.9%        |                |
| P-value      | 1.21E-5 <0.0001     |                |

The analysis results from this population were less conservative than the sponsor’s primary analysis considering the survival rate in the levofloxacin group and the lower limit of the confidence interval. Therefore, it is acceptable to exclude these two animals from the primary analysis population.

However, if animal X717 was considered as a non-survivor and X779 as a survivor, the difference in survival proportions and the lower limit of its 95% confidence interval would be lower than those from the sponsor’s primary analysis, as the following table shows. Thus, this analysis results were more conservative. However, the results continue to be highly significant and there is a convincing rationale to support the removal of animal X717 given that it was not challenged.

*Comment: Note that a slight concern remains regarding whether or not this ill animal would have been removed from the study if it had been randomized to the placebo arm. This points to*

*the problems with conducting a study that is not completely blinded. We can never know if the decision to remove this animal was affected by knowledge of its treatment assignment.*

**Table 8: Survival at Day 28 post exposure for all randomized animals (X717 as non-survivor but X779 as survivor), ITT population**

|              | <b>Levofloxacin</b> | <b>Placebo</b> |
|--------------|---------------------|----------------|
| Survival (%) | 17/19 (89.5%)       | 0/7            |
| 95% CI       | 42.1%, 98.7%        |                |
| P-value      | 5.47E-5 <0.0001     |                |

A conservative analysis regarding these two animals was conducted considering both animals as non-survivors. The results of this analysis are reported in the following table.

**Table 9: Survival at Day 28 post exposure for all randomized animals (both X717 and X779 as non-survivors), ITT population**

|              | <b>Levofloxacin</b>      | <b>Placebo</b> |
|--------------|--------------------------|----------------|
| Survival (%) | 16/19 (84.2%)            | 0/7            |
| 95% CI       | 42.1%, 96.6%             |                |
| P-value      | 1.82E-4 $\approx$ 0.0002 |                |

This is the most conservative analysis for the ITT population. The difference in survival proportions remained statistically significant.

Given these considerations, the remaining analyses will exclude these two animals.

***FDA’s survival analysis for Cohorts 1 and 2***

This was an open-label study and cohort 3 was added about 7 months after the completion of Cohort 2. It might be argued that the study should be assessed in a stepwise fashion. That is, look at cohorts 1 and 2 first and if the results are significant, go on to the analysis including cohorts 1, 2 and 3.

*Comment: An additional reason for conducting an analysis without cohort 3 is that as shown in Figure 3 the challenge doses were much lower in this cohort and a number of animals had LD<sub>50</sub>s lower than 20 LD<sub>50</sub>s, below which untreated animals survived in a natural history study (see appendix). Therefore, an analysis of cohorts 1 and 2 only is of interest to address this concern as well. Note that an additional analysis excluding only animals with LD<sub>50</sub>s less than 20 was also conducted below.*

The data from Cohorts 1 and 2 show a statistically significant difference in survival up to day 28 between the two groups (9/9 versus 0/5, Fisher’s exact one-sided p-value =0.0005). Since the difference in survival proportion was statistically significant, it is acceptable to include the 3<sup>rd</sup> cohort.

**Table 10: Survival at Day 28 post exposure for Animals from Cohorts 1 and 2, per protocol population**

|              | <b>Levofloxacin</b> | <b>Placebo</b> |
|--------------|---------------------|----------------|
| Survival (%) | 9/9 (100%)          | 0/5            |
| 95% CI       | 47.4%, 100%         |                |
| P-value      | 0.0005              |                |

### **Additional Sensitivity survival analyses**

#### *Survival for animals from three cohorts with bacteremia at initiation of treatment*

Six animals in the levofloxacin group and two control animals (X773 and X888) did not have bacteremia at the initiation of treatment were excluded from this sensitivity analysis. Note that the two controls became bacteremic the day after the first infusion. The following table shows the results of an analysis that removes these 8 animals. There continues to be a statistically significant difference in survival between the two treatment groups.

**Table 11: Survival at Day 28 post exposure for animals from three cohorts with bacteremia at start of treatment**

|              | <b>Levofloxacin</b> | <b>Placebo</b> |
|--------------|---------------------|----------------|
| Survival (%) | 11/12 (91.7%)       | 0/5            |
| 95% CI       | 28.0%, 99.8%        |                |
| P-value      | 0.0010              |                |

#### *Survival for Cohorts 1 and 2 animals with pulmonary infiltrates*

All animals in Cohort 1 and all but 2 animals in Cohort 2 had Day-5 chest radiograph information available (these 2 animals in the control group had died or were euthanized before chest radiograph was taken). Note that no animals in cohort 3 had chest radiographs taken post challenge. According to the medical review, there were 9 and 3 animals in the two groups with pulmonary infiltrates. The following table shows the comparison of survival for these animals. Still there was a significant difference between the two treatment groups (Fisher's exact p-value 0.005).

**Table 12: Survival for Cohorts 1 and 2 with pulmonary infiltrates on chest radiograph, excluding animal X779**

|              | <b>Levofloxacin</b> | <b>Placebo</b> |
|--------------|---------------------|----------------|
| Survival (%) | 9/9 (100%)          | 0/3            |
| 95% CI       | 29.0%, 100%         |                |
| P-value      | 0.0045              |                |

### ***Survival for animals with LD<sub>50</sub> greater than 20***

Six animals from Cohort 3 (including the non-survivor Y160) received less than 20 LD<sub>50</sub>s. As discussed previously, based on the results from natural history studies, the efficacy results from an analysis for animals with LD<sub>50</sub> greater than 20 should be examined. The following table shows the survival analysis for these animals. There continues to be a statistically significant difference in survival proportions between the two groups.

**Table 13: Survival for Cohorts 1 and 2 with greater than 20 LD<sub>50</sub>s, excluding animal X779**

|              | <b>Levofloxacin</b> | <b>Placebo</b> |
|--------------|---------------------|----------------|
| Survival (%) | 12/12 (100%)        | 0/7            |
| 95% CI       | 59.0%, 100%         |                |
| P-value      | <0.0001             |                |

Note that the two animals in the natural history studies with LD<sub>50</sub>s < 20 did not have any signs of disease, while the animals in the current efficacy study did show signs of disease including fever and bacteremia.

### ***Microbiological cure***

As mentioned briefly previously, three levofloxacin treated animals had blood cultures and/or lung tissue culture positive for *Y. pestis* at study termination on Day 28: one animal had a positive blood culture only; one animal had a positive lung tissue culture only; one animal had positive cultures in blood and lungs. All three animals had negative blood cultures for *Y. pestis* on days 7 through Day 14. The following table shows a statistical significant difference in microbiological cure rates between the two groups.

**Table 14: Microbiological cure at Day 28 in 3 cohorts**

|              | <b>Levofloxacin</b> | <b>Placebo</b> |
|--------------|---------------------|----------------|
| Survival (%) | 14/17 (82.3%)       | 0/7            |
| 95% CI       | 29.0%, 96.3%        |                |
| P-value      | 0.0003              |                |

### **Efficacy conclusions**

Based on the sponsor's efficacy analysis as well as all of FDA's sensitivity analyses, there was a statistically significant difference in proportion surviving to Day 28 after challenge between the two treatment groups. Therefore, the study provided adequate evidence for the efficacy of levofloxacin in the treatment of pneumonic plague in AGMs.

### **3.3 Evaluation of Safety**

This study was an animal study for evaluation of efficacy. The safety evaluation of levofloxacin in human relies on previous safety data for the NDA application.

### 3.4 Benefit:Risk Assessment

Not conducted.

## 4 FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

### 4.1 Gender, Race, Age, and Geographic Region

Note that since the sample size is small, there was only one death in the levofloxacin group and all animals in the control group did not survive, subgroup analyses are of limited utility.

An analysis by age was not conducted because the actual ages of the animals were not known. The survival by gender for animals from the three cohorts is as follows:

**Table 15: Survival at Day 28 post exposure for animals from three cohorts by gender**

| <b>Gender</b> | <b>Levofloxacin</b> | <b>Placebo</b> |
|---------------|---------------------|----------------|
| Male          | 8/8 (100%)          | 0/4            |
| Female        | 8/9 (88.9%)         | 0/3            |

The non-surviving animal was female. Therefore, the survival proportion in the levofloxacin group among female AGMs was lower than 100%. Additional analyses were conducted to see if there were any differences in LD<sub>50</sub> dose, time to fever, or time to death (in controls) between gender. No differences were seen.

**Table 16: Summary of challenge dose, time to fever, and time to death by gender**

|  | <b>Male</b>  | <b>Female</b> |
|--|--------------|---------------|
| <b>LD<sub>50</sub></b>                       | N=12         | N=12          |
| Mean   | 69           | 61            |
| SD   | 53           | 44            |
| Range  | 3-145        | 3-124         |
| <b>Time to fever (hrs)</b>                   | N=12         | N=12          |
| Mean   | 82.3         | 73.6          |
| SD   | 32.0         | 18.5          |
| Range  | 52.8 – 165.4 | 58.6 – 125.0  |
| <b>Time to death (hr) in control animals</b> | N=4          | N=3           |
| Mean   | 107.0        | 104.7         |
| SD   | 15.2         | 16.9          |
| Range  | 86.4-122.1   | 85.9-118.8    |

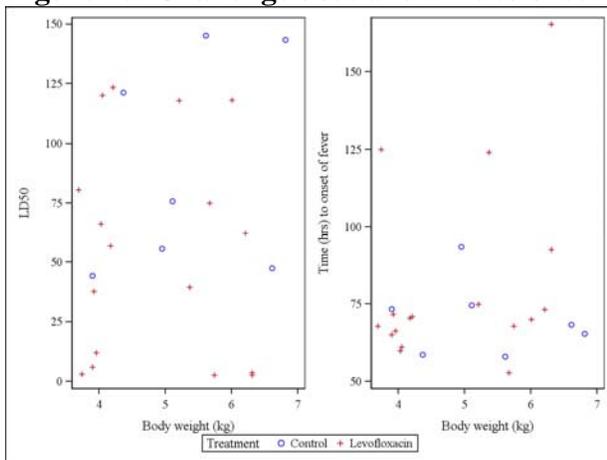
Race and geographic region are not applicable.

## 4.2 Other Special/Subgroup Populations

### 4.2.1 Challenge dose, time to fever, and time to death by body weight

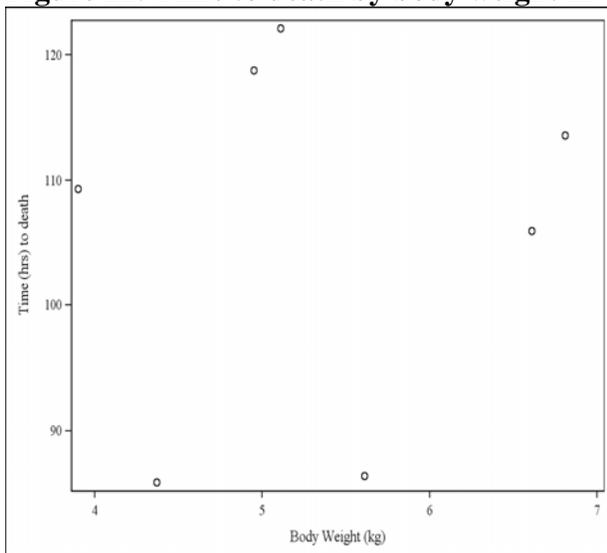
The only additional baseline characteristic that is available for the animals from this study is weight. For completion analyses were conducted by the weight of the animals to see if any patterns were seen. The following graphs show LD<sub>50</sub>s by weight (kg) and time to onset of fever (hrs) since challenge by weight. There was no clear pattern that weight was associated with these two variables.

**Figure 10: Challenge dose and time to onset of fever by body weight**



The following graph shows the time to death (hrs) by body weight (kg) in control animals. Body weight was not associated with the time to death.

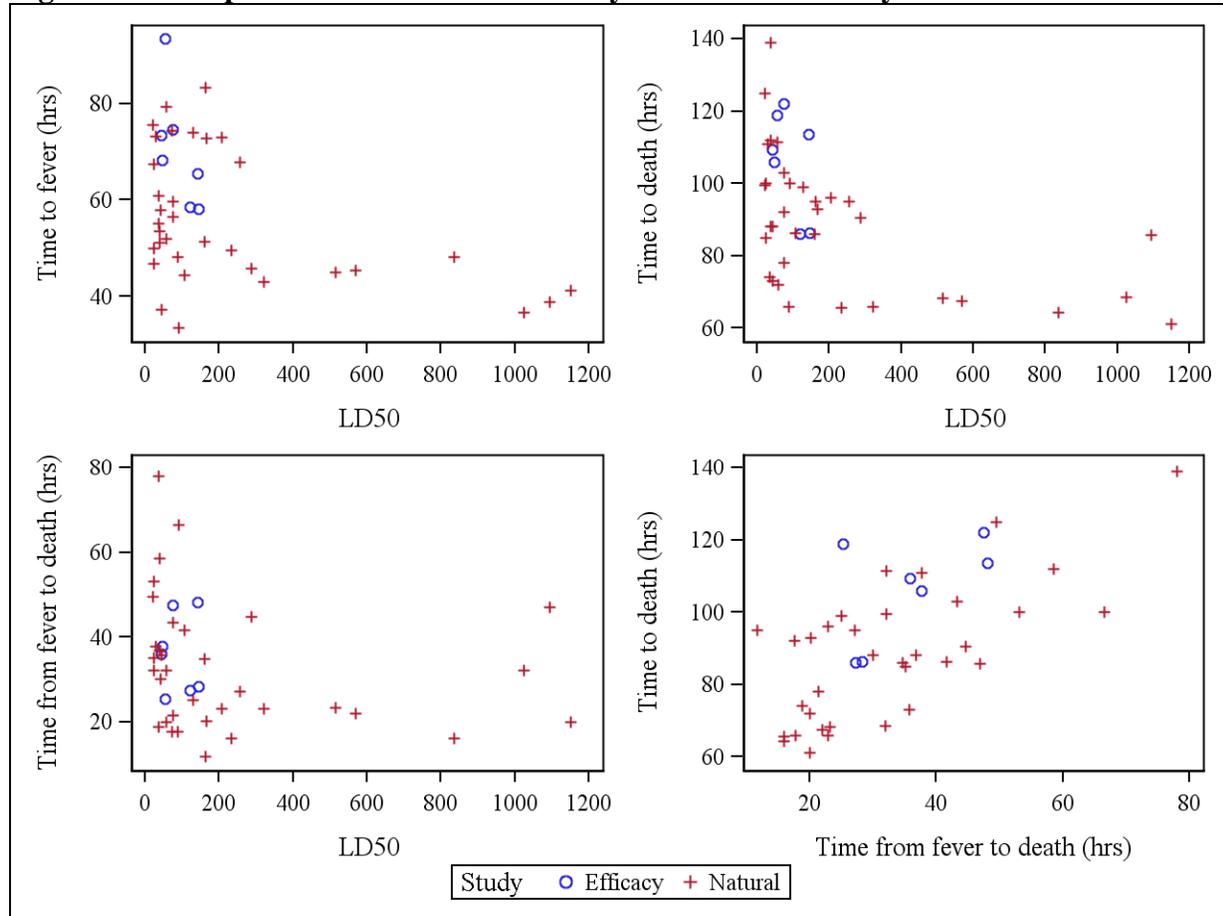
**Figure 11: Time to death by body weight in control animals**



## Comparison of control animals with animals from the natural history studies

There were 7 controls in current efficacy study. There were 34 animals in the four natural history studies. The following graphs show the comparison of these controls from the efficacy study and the four natural history studies. The 7 controls from the efficacy study were well scattered among these controls with similar challenge doses. Time to death and time to fever were similar to those from the natural history studies.

**Figure 12: Comparison of controls in efficacy and natural history studies**



## 5 SUMMARY AND CONCLUSIONS

### 5.1 Statistical Issues and Collective Evidence

Twenty-six animals were enrolled, 16 of which were enrolled in the first two cohorts as planned in the initial protocol. One animal in Cohort 2 (X717) was randomized to levofloxacin but removed from the study due to health reasons prior to challenge. One animal in Cohort 2 in the levofloxacin group (X779) was challenged but received the first treatment prior to onset of fever when it was supposed to receive treatment after onset of fever and a partial dose on Day 9. This animal survived without additional treatment.

The trial was an open-label study, although the principle investigator making euthanasia decisions was blind to the assignment of treatment group. Therefore, we were cautious when considering the two animals that were excluded from the sponsor's primary analysis. We compared the survival proportions between the two treatment groups in ITT populations, assuming different scenarios for the two animals excluded in the sponsor's primary analysis. The results remained highly significant for all analyses.

Cohort 3 of sample size 10 was added a few months later after the completion of Cohort 2 and most likely after the data from cohorts 1 and 2 were analyzed. For this reason, we considered it important to consider the results of the study in a stepwise fashion, i.e., consider the results of cohorts 1 and 2 alone and if significant, include the third cohort. The results of the first two cohorts were significant, so we felt comfortable considering all three cohorts in our analysis.

Additional sensitivity analyses on survival and microbiological response also showed consistently significant differences between the two treatment groups. These results support the efficacy of levofloxacin in the treatment of pneumonic plague after exposure to *Y. pestis* in AGMs.

The primary efficacy endpoint was survival up to Day 28, not clinical cure or microbiological cure. Therefore, the two animals with positive culture results were considered as survivors. This distinction might be noted in the label to remind physicians that the efficacy was based on survival, not on clinical and/or microbiological cure.

Safety evaluation relies on safety data from other approved indications. If the proposed dosage for this indication is not greater than the dosages for other approved indications, there is no concern for safety.

## **5.2 Conclusions and Recommendations**

The efficacy of levofloxacin in the treatment of pneumonic plague *due to Y. pestis* in AGMs was supported by this animal study. Whether or not the efficacy results can be a reliable indicator of its effectiveness in the treatment of human pneumonic plague depends upon the preclinical and clinical evaluation based on the Animal Rule.

There is no safety concern if the proposed dosage for this condition in humans is not higher than the dosages for other approved conditions for levofloxacin.

## 6 APPENDIX

### Natural History Studies in African Green Monkeys

Four natural history studies were conducted to demonstrate that the AGMs model is an appropriate animal model for pneumonic plague. The study numbers, doses, sample sizes, number of deaths, and fever definitions from these studies are summarized in the following table.

**Table 17: Summary of pneumonic plague in natural history studies**

| Study                | Dose LD <sub>50</sub> | N  | Number of death | Fever definition   |
|----------------------|-----------------------|----|-----------------|--|
| F03-09G USAMRIID     | 9-57                  | 6  | 4               | ≥1.5°C above baseline (baseline was not defined).                                |
| Fy06-126 Lovelace    | 44-255                | 10 | 10              | 1 hr average > 39°C*   |
| 617-g607610 Battelle | 106-1150              | 10 | 10              | 3 consecutive hourly measurements at least 1.5°C above baseline at the same hour |
| 875-g607610 Battelle | 24-88                 | 10 | 10              | >1.5°C higher than baseline average (baseline average was not defined)           |

\*Above 1 and 2°C above baseline were compared.

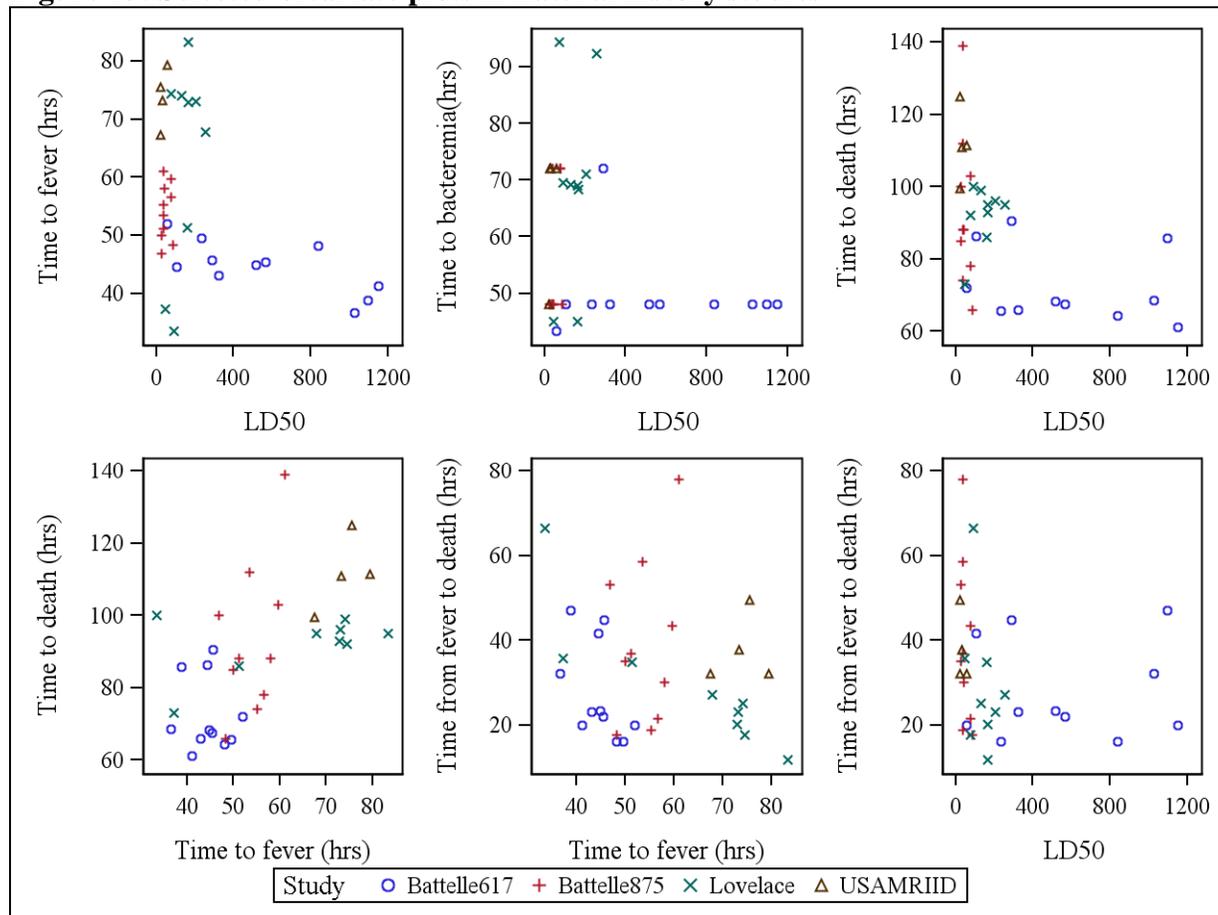
When baseline was not well defined, we used 24 hourly matched averages as baseline.

Four of the 6 challenged animals in Study F03-09G, receiving a challenge doses more than 20 LD<sub>50</sub>s died due to pneumonic plague. The other 2 animals with a dose of 9 LD<sub>50</sub>s (or 3100 CFU) and 12 (or 4300 CFU) survived without any clinical signs or bacteremia. The 2 survivors received 0.2 and 0.3 LD<sub>99</sub>s respectively. All animals from the other three natural history studies died within 4 days of challenge and all received doses greater than 20 LD<sub>50</sub>s.

*Comment: From this natural history study, low doses of less than 12 LD<sub>50</sub>s were not lethal. There were no animals that received a challenge dose between 12 and 20 LD<sub>50</sub>s from all the natural history studies. Therefore, in the efficacy study, if the challenge dose was less than 20 LD<sub>50</sub>s, caution is needed to interpret the survival outcome.*

The following figure shows selected bivariate plots in natural history studies. Time to onset of fever in these studies versus LD<sub>50</sub> challenge dose is shown in the first graph in the first row. On average, lower challenge doses were associated with longer time to onset of fever (the Pearson correlation coefficient was -0.449, p-value=0.0077; the Spearman correlation coefficient was -0.438, p-value=0.0095). When the dose was less than 300 LD<sub>50</sub>s, there was a considerable variability in time to onset of fever. However, once the dose was greater than 300 LD<sub>50</sub>s, time to onset of fever was stabilized and less than 50 hours.

**Figure 13: Selected bivariate plots in natural history studies**



The second graph in the first row shows time to bacteremia versus challenge dose. As challenge dose reached 300 LD<sub>50</sub>s, all animals became bacteremic within 48 hours.

The third graph in the first row shows time to death versus challenge dose. When challenge dose was less than 300 LD<sub>50</sub>s, there was a considerable variability in the time to death. Overall, there was a negative association between time to death and challenge dose (the Pearson correlation coefficient was -0.503, p-value=0.0024; the Spearman correlation coefficient -0.577, p-value=0.0004).

The first graph in the second row shows the time from challenge to death and time to fever. It seems that longer time to fever was associated with longer time to death because time from challenge to death included time to fever. The Pearson correlation coefficient was 0.575, p-value=0.0004. The Spearman correlation coefficient was 0.607, p-value=0.0001.

As the second graph in the second row shows, time from onset of fever to death appears not correlated with time from challenge to fever (the Pearson correlation coefficient was -0.200, p-

value=0.257; the Spearman correlation coefficient -0.154, p-value=0.386). Once animals became febrile, the time from onset of fever to death was around 40 hours.

The third graph in the second row shows time from onset of fever to death versus challenge dose. These two variables were not well linearly correlated. The Pearson correlation coefficient was -0.204, p-value=0.248. The Spearman correlation coefficient was -0.392, p-value=0.022. In addition, there was a considerable variability in time from fever to death, especially when the dose was less than 300 LD<sub>50</sub>s. Note that the relationship between time to death versus challenge dose is stronger than the relationship between time from fever to death versus challenge dose. Since the studies are designed to treat animals at the time of fever, this lessens the impact of the specific challenge dose on the animal's outcome.

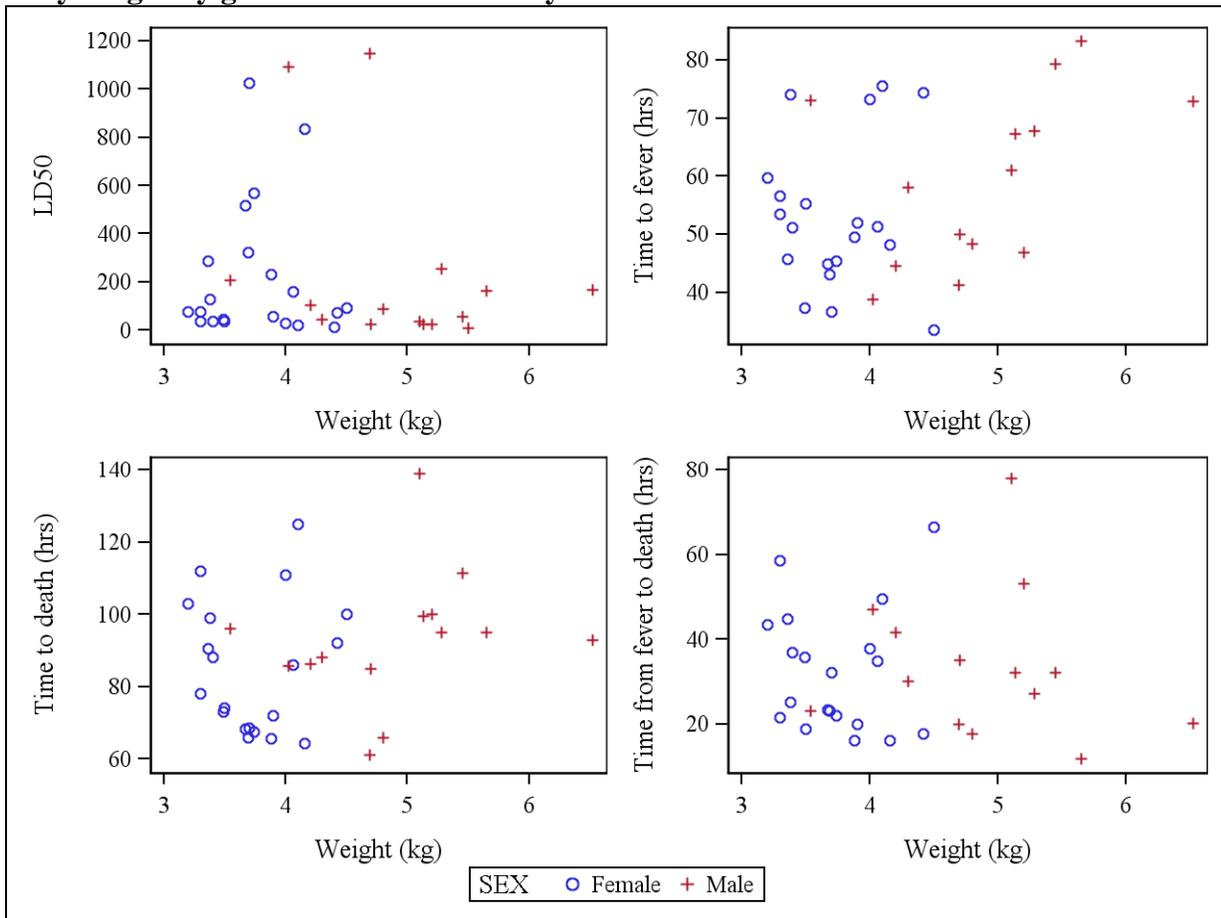
We also conducted additional analyses by gender and body weight. The following table shows the summary of challenge dose, time to fever, and time to death, by gender in the natural history studies. No large differences were seen in these variables between male and female animals.

**Table 18: Summary of challenge dose, time to fever, and time to death by gender in natural history studies**

|                            | <b>Male</b><br>N=15 | <b>Female</b><br>N=21 |
|----------------------------|---------------------|-----------------------|
| <b>LD<sub>50</sub></b>     |                     |                       |
| Mean                       | 229.8               | 222.3                 |
| SD                         | 370.0               | 284.4                 |
| Range                      | 9 – 1150            | 12 – 1024             |
| <b>Time to fever (hrs)</b> |                     |                       |
| Mean                       | 59.4                | 53.0                  |
| SD                         | 14.7                | 12.8                  |
| Range                      | 38,8 – 83.3         | 33.5 – 75.5           |
| <b>Time to death (hrs)</b> |                     |                       |
| Mean                       | 92.9                | 85.2                  |
| SD                         | 18.6                | 18.3                  |
| Range                      | 61.2 – 139.0        | 64.2 – 125.0          |

The following figure shows challenge dose, time to fever, time to death, and time from fever to death, versus body weight by gender. Although male animals were heavier than females, there were no discernible differences in these variables as body weight changes, except that some animals with low body weight received higher challenge doses.

**Figure 14: Challenge dose, time to fever, time to death, time from fever to death, versus body weight by gender in natural history studies**



For information on the sign and symptoms of plague in these animals, see medical officer's review. It was concluded that the animal model is an appropriate model for pneumonic plague.

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/s/  
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XIANBIN LI  
04/06/2012

KAREN M HIGGINS  
04/06/2012  
I concur.

## STATISTICS FILING CHECKLIST FOR A NEW NDA/BLA

**NDA Number:** N20634      **Applicant:** Johnson & Johnson PRD      **Stamp Date:** 10/28/2011

**Drug Name:** Levaquin      **NDA/BLA Type:** Supplemental NDA

On **initial** overview of the NDA/BLA application for RTF:

|   | <b>Content Parameter</b>  | <b>Yes</b> | <b>No</b> | <b>NA</b> | <b>Comments</b>  |
|---|---|------------|-----------|-----------|--|
| 1 | Index is sufficient to locate necessary reports, tables, data, etc.   | X          |           |           |  |
| 2 | ISS, ISE, and complete study reports are available (including original protocols, subsequent amendments, etc.)                  | X          |           |           |  |
| 3 | Safety and efficacy were investigated for gender, racial, and geriatric subgroups investigated (if applicable).                 |            |           | X         | Efficacy is determined from an animal study. Race is not applicable. Gender and age will be reviewed but extrapolating results to humans might be difficult. The drug is approved and safety in these subgroups previously assessed. |
| 4 | Data sets in EDR are accessible and do they conform to applicable guidances (e.g., existence of define.pdf file for data sets). | X          |           |           |  |

**IS THE STATISTICAL SECTION OF THE APPLICATION FILEABLE?** \_\_\_ Yes \_\_\_

If the NDA/BLA is not fileable from the statistical 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.

| <b>Content Parameter (possible review concerns for 74-day letter)</b>   | <b>Yes</b> | <b>No</b> | <b>NA</b> | <b>Comment</b> |
|---|------------|-----------|-----------|----------------|
| Designs utilized are appropriate for the indications requested.   | X          |           |           |                |
| Endpoints and methods of analysis are specified in the protocols/statistical analysis plans.  | X          |           |           |                |
| Interim analyses (if present) were pre-specified in the protocol and appropriate adjustments in significance level made. DSMB meeting minutes and data are available. |            |           | X         |                |
| Appropriate references for novel statistical methodology (if present) are included.   |            |           | X         |                |

## STATISTICS FILING CHECKLIST FOR A NEW NDA/BLA

|   |  |  |   |  |
|---|--|--|---|--|
| Safety data organized to permit analyses across clinical trials in the NDA/BLA.                         |  |  | X |  |
| Investigation of effect of dropouts on statistical analyses as described by applicant appears adequate. |  |  | X |  |

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/s/  
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XIANBIN LI  
11/21/2011

KAREN M HIGGINS  
11/21/2011

## **STATISTICAL IND/NDA REVIEW AND EVALUATION**

**#IND and #NDA:** N20634: N20635, N21721, IND 36627, IND 38368  
**Submission #:** N20634: SN 63 & 64, SDN 485 & 486;  
N20635: SN 65 & 66, SDN 495 & 496;  
N21721: SN 63 & 64, SDN 199 & 200;  
IND 36627: SN 686 & 688, SDN 873 & 875;  
IND 38368: SN 547 & 549, SDN 644 & 646  
(4/29/11, 5/20/2011)  
**Name of Drug:** Levaquin (levofloxacin)  
**Sponsor:** Johnson & Johnson Pharmaceutical Research & Development,  
L.L.C.  
**Indications:** Plague  
**Statistical Reviewer:** Xianbin Li  
**Medical Reviewer:** Elizabeth O'Shaughnessy  
**Project Manager:** Jane Dean

These two submissions contain mock data set definitions for plague natural history studies and excel spreadsheet containing a mock data set. After reviewing the first submission, FDA sent comments on 5/10/2011. Since the data set format is acceptable, no statistical comment was sent then.

In the cover letter in the second submission, the sponsor wanted to clarify that the mock data set and data definitions submitted in the first submission followed the CDISC format. Additionally, the raw telemetry data is obtained in a file for each animal, with rows for time points and columns for the parameters (temperature, heart rate, etc.). The sponsor wanted to present the telemetry data in this alternative format.

After reviewing the sample data, we sent the following comment to the sponsor on 6/6/2011:

The mock data set and data definitions submitted on 29 April 2011 followed the CDISC format where each data point is represented by a row in the SAS data set. Although the alternative telemetry data format does not follow the CDISC format, it is acceptable. As discussed before, please submit all data in SAS transport data format.

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/s/  
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XIANBIN LI  
06/13/2011

KAREN M HIGGINS  
06/15/2011