BRIEFING PACKAGE

Division of Anti-Infective Products

Office of Antimicrobial Products

Center for Drug Evaluation and Research, FDA

ANTI-INFECTIVE DRUGS ADVISORY COMMITTEE MEETING

APRIL 3, 2012 – MORNING SESSION

TOPIC:

AFRICAN GREEN MONKEY (*CHLOROCEBUS AETHIOPS*) ANIMAL MODEL DEVELOPMENT TO EVALUATE TREATMENT OF PNEUMONIC PLAGUE

ACKNOWLEDGEMENT

This briefing document was prepared jointly by the Division of Anti-Infective Products (DAIP), Center for Drug Evaluation and Research (CDER), FDA and the Division of Microbiology and Infectious Diseases (DMID) of the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health. DMID wrote the medical literature summary on human pneumonic plague and summarized the natural history studies of pneumonic plague in African Green Monkeys. The staff of DAIP is grateful to the staff of DMID for their major contributions to this document.

DISCLAIMER STATEMENT

The attached package contains background information prepared, in part, by the Food and Drug Administration (FDA) for the panel members of the advisory committee. The FDA background package often contains assessments and/or conclusions and recommendations written by individual FDA reviewers. Such conclusions and recommendations do not necessarily represent the final position of the individual reviewers, nor do they necessarily represent the final position of the Review Division or Office. We have brought this issue to this Advisory Committee in order to gain the Committee's insights and opinions, and the background package may not include all issues relevant to the final regulatory recommendation and instead is intended to focus on issues identified by the Agency for discussion by the advisory committee. The FDA will not issue a final determination on the issues at hand until input from the advisory committee process has been considered and all reviews have been finalized. The final determination may be affected by issues not discussed at the advisory committee meeting.

Table of Contents

LIST OF IN-TEXT TABLES **4**

LIST OF IN-TEXT FIGURES **4**

LIST OF APPENDICES **4**

ABBREVIATIONS **5**

EXECUTIVE SUMMARY **6**

1 INTRODUCTION **7**

1.1 ANIMAL RULE 7

1.1.1 Criteria for Submission *8*

1.1.2 Criteria for Approval *8*

1.2 REGULATORY HISTORY OF PNEUMONIC PLAGUE ANIMAL MODEL DEVELOPMENT 10

2 PNEUMONIC PLAGUE **10**

2.1 HUMAN CLINICAL DISEASE 10

2.2 MORBIDITY AND MORTALITY ASSOCIATED WITH PNEUMONIC PLAGUE 12

2.2.1 1905 Treatise on Plague *12*

2.2.2 1924 Los Angeles Epidemic *14*

2.2.3 1926 Treatise on Pneumonic Plague *14*

2.2.4 Plague: a manual for medical and public health workers *16*

2.2.5 Radiographic Findings in Untreated Pneumonic Plague *21*

2.2.6 Summary of Untreated Clinical Course of Pneumonic Plague *21*

2.3 ANTIBACTERIAL DRUG TREATMENT OF PNEUMONIC PLAGUE DESCRIBED IN MEDICAL LITERATURE 21

2.3.1 Treatment with Sulfadiazine and Penicillin *21*

2.3.2 Treatment with Sulfadiazine, Sulfamerzine, and Streptomycin *22*

2.3.3 Treatment with Tetracycline and Streptomycin *24*

2.3.4 Treatment with Chloramphenicol and Doxycycline *25*

2.3.5 Summary of Treated Clinical Course of Pneumonic Plague in Medical Literature *26*

3 THE AFRICAN GREEN MONKEY (AGM) MODEL OF PNEUMONIC PLAGUE **26**

3.1 DETERMINATION OF THE MEDIAN LETHAL DOSE OF *YERSINIA PESTIS* IN AFRICAN GREEN MONKEYS 28

3.2 NATURAL HISTORY OF PNEUMONIC PLAGUE IN AFRICAN GREEN MONKEYS 29

3.2.1 Natural History Study of Pneumonic Plague in the African Green Monkey (Study No.F03-09G-Phase-1) *29*

3.2.2 Natural History Study of Inhalational Plague Y. pestis Strain CO92 in Adult Telemetered African Green Monkeys (Study No. FY06-126) *33*

3.2.3 Natural Course of Untreated Pneumonic Plague in African Green Monkeys (Study No. 617-G607610) *36*

3.2.4 Natural Course of Untreated Pneumonic Plague in African Green Monkeys (Study No. 875-G607610) *40*

3.3 SUMMARY OF THE AFRICAN GREEN MONKEY (AGM) MODEL OF PNEUMONIC PLAGUE 43

4 COMPARISON OF HUMAN AND ANIMAL (AFRICAN GREEN MONKEY) NATURAL COURSES OF PNEUMONIC PLAGUE – DMID PERSPECTIVE **43**

5 PNEUMONIC PLAGUE – NATURAL HISTORY IN AFRICAN GREEN MONKEY (AGM) MODEL AND COMPARISON TO HUMAN DISEASE – DAIP PERSPECTIVE 46

5.1 NATURAL HISTORY STUDIES OF PNEUMONIC PLAGUE IN AFRICAN GREEN MONKEYS 46

5.2 COMPARISON OF AGM MODEL OF PNEUMONIC PLAGUE TO HUMAN DISEASE 48

6 POINTS FOR DISCUSSION 52

7 REFERENCES 53

8 APPENDICES 55

List of In-text Tables

TABLE 1 F03-09G-PHASE-1 NATURAL HISTORY: CHALLENGE DOSE, SURVIVAL, FEVER, BACTEREMIA OBSERVATIONS 32

TABLE 2 F03-09G-PHASE-1 NATURAL HISTORY: PROMINENT GROSS LESIONS 32

TABLE 3 F03-09G-PHASE-1 NATURAL HISTORY: MICROSCOPIC FINDINGS 33

TABLE 4 FY06-126 NATURAL HISTORY: CHALLENGE DOSE, SURVIVAL, FEVER AND BACTEREMIA OBSERVATIONS 35

TABLE 5 FY06-126 NATURAL HISTORY: SUMMARY OF PROMINENT MICROSCOPIC FINDINGS 36

TABLE 6 617-G607610 NATURAL HISTORY: CHALLENGE DOSE, SURVIVAL, FEVER AND BACTEREMIA OBSERVATIONS 38

TABLE 7 617-G607610 NATURAL HISTORY: SUMMARY OF PROMINENT GROSS AND MICROSCOPIC FINDINGS 39

TABLE 8 875-G607610 NATURAL HISTORY: CHALLENGE DOSE, SURVIVAL, FEVER AND BACTEREMIA OBSERVATIONS 41

TABLE 9 875-G607610 NATURAL HISTORY: SUMMARY OF PROMINENT PATHOLOGIC FINDINGS 42

TABLE 10 HUMAN AND AFRICAN GREEN MONKEY NATURAL COURSES OF PNEUMONIC PLAGUE 45

TABLE 11 SUMMARY FINDINGS, AGM PRIMARY PNEUMONIC PLAGUE NATURAL HISTORY STUDIES 47

TABLE 12 INTERSPECIES COMPARISON 50

List of In-text Figures

FIGURE 1 PROVENANCE OF *Y. PESTIS* CO92 27

List of Appendices

APPENDIX A INDEPENDENT PATHOLOGY REVIEW SUMMARY 55

APPENDIX B INDEPENDENT RADIOLOGY REVIEW SUMMARY 67

ABBREVIATIONS

AGM	African Green Monkey
BBRC	Battelle Biomedical Research Center
bpm	beats per minute
brpm	breaths per minute
BSGT	buffered saline with gelatin and trehalose
C	Celsius
CDC	United States Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CFU	colony forming units
cm	Centimeter
DAIP	Division of Anti-Infective Products
DMID	Division of Microbiology and Infectious Diseases
ELISA	enzyme-linked immunosorbent assay
F	Female
F	Fahrenheit
FDA	United States Food and Drug Administration
g	gram(s)
GLP	Good Laboratory Practices
h	Hour
HIB	heart infusion broth
IM	Intramuscular
IND	Investigational New Drug Application
IP	Intraperitoneal
IV	Intravenous
kg	Kilogram
LD_{50}	median lethal dose
LD_{99}	dose required for 99% lethality
LRRI	Lovelace Respiratory Research Institute
M	Male
mL	Milliliter
NA	not applicable
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
nm	Nanometers
NR	not reported
PIND	Pre-Investigational New Drug Application
Pgm	Pigmentation locus in *Yersinia pestis*
PO	per os (orally)
SD	standard deviation
SOP	standard operating procedure
Std dev	standard deviation
TBAB	tryptose blood agar base
TSA	tryptic soy agar
UNM	University of New Mexico
US	United States
USAMRIID	United States Army Medical Research Institute of Infectious Diseases
Y. pestis	*Yersinia pestis*

Executive Summary

This briefing document was prepared jointly by the Division of Anti-Infective Products (DAIP), Center for Drug Evaluation and Research (CDER), FDA and the Division of Microbiology and Infectious Diseases (DMID) of the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health. The document is intended to provide information in preparation for the Center for Drug Evaluation and Research Anti-Infective Drugs Advisory Committee Meeting on April 3, 2012, during which DMID, at the request of DAIP, will make presentations to discuss:

- Clinical Course of Untreated Pneumonic Plague
- Clinical Course of Pneumonic Plague Treated with Early Generation Antibacterial Drugs
- Natural Course of Untreated Pneumonic Plague in African Green Monkeys
- Comparison of Human and Animal (AGM) Natural Course of Pneumonic Plague

Pneumonic plague as a naturally occurring disease is extremely rare in the United States as well as in endemic areas worldwide, but is the likely disease manifestation if *Yersinia pestis* is employed as a biological weapon. As naturally-occurring pneumonic plague in humans is exceedingly rare, evaluation of efficacy for products against pneumonic plague will have to be conducted in animal models of infection under a regulatory mechanism known as the Animal Rule. In an attempt to establish a platform for evaluating products for the treatment of pneumonic plague DMID/NIAID/NIH undertook the development of an animal model to evaluate drugs for the treatment of pneumonic plague.

1 Introduction

The Division of Anti-Infective Products of the United States (US) Food and Drug Administration (FDA) is convening an Advisory Committee meeting on April 3, 2012 to discuss regulatory considerations surrounding the African Green Monkey (AGM) model of pneumonic plague. The DAIP has requested a meeting for which DMID has prepared portions of this briefing document addressing the following:

- Clinical Course of Untreated Pneumonic Plague (Section 2)
- Clinical Course of Pneumonic Plague Treated with Early Generation Antibacterial Drugs (Section 2)
- Natural Course of Untreated Pneumonic Plague in African Green Monkeys (Section 3)
- Comparison of Human and Animal (AGM) Natural Course of Pneumonic Plague (Section 4)

1.1 Animal Rule

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

To address this situation, and provide a regulatory mechanism to accept efficacy from such animal models, FDA published a Final Rule in 2002 titled "New Drug and Biological Drug Products; Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible,"[1] that is also referred to as the "Animal Rule." As noted above, this rule applies when adequate and well-controlled studies in humans cannot be ethically conducted and field efficacy studies are not feasible. In such circumstances, products intended to reduce or prevent serious or life-threatening conditions may be approved for marketing based on

[1] Final Rule published in the Federal Register, Vol. 67, No. 105, May 31, 2002, pages 37988-37998; Regulations: 21 CFR § 314.600-650 (New Drugs), 21 CFR § 601.90-95 (Biologics)

evidence of effectiveness derived from appropriate studies in animals and additional supporting data.

This rule is codified in the Code of Federal Regulations (CFR), in Title 21 CFR 314.600 (Subpart I) for New Drugs and in Title 21 CFR 601.90 for Biologic Products. Excerpts and summaries from the Final Rule and the CFR are provided below and describe the type of evidence and information that need to be provided to the FDA for determination of efficacy.

1.1.1 Criteria for Submission

Because pneumonic plague is an extremely rare disease in humans and intentional exposure is not ethical, efficacy of new products for the treatment of pneumonic plague need to be derived from studies of animal models of infection. In January 2009, FDA published a draft *Guidance for Industry: Animal Models – Essential Elements to Address Efficacy Under the Animal Rule*,[2] which provides information on the development of animal models to study efficacy, including the critical characteristics of an animal model that need to be addressed under the Animal Rule. For example, these elements include the characteristics of the agent that causes the disease (in this case *Y. pestis*), the host susceptibility and response to the agent, the natural history of the disease in humans and comparability to animal models, the trigger and timing of intervention, the characteristics of the medical intervention, and study design considerations.

All animal studies subject to this rule must be conducted in accordance with Good Laboratory Practices (GLP) requirements.

Safety evaluation of products is not addressed in the Animal Rule. Products evaluated for effectiveness under Subpart I of part 314 for drugs are expected to be evaluated for safety under preexisting requirements for establishing the safety of drugs. FDA believes that the safety of most of these products can be studied in human volunteers similar to those who could potentially be exposed to the product. FDA recognizes that some safety data, such as data on possible adverse interactions between the toxic substance itself and the drug, may not be available.

1.1.2 Criteria for Approval

The Animal Rule states that a drug can be approved on the basis of adequate and well-controlled animal studies when the results of those animal studies establish that the study drug is reasonably likely to produce clinical benefit in humans. In assessing the sufficiency of animal data, FDA may take into account other data, including human data, available to the Agency. FDA will rely on the evidence from studies in animals to provide substantial evidence of the effectiveness of these products only when:

- There is a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product;

[2] http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm078923.pdf

- The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans;
- The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity; and
- The data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.

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

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

1. Postmarketing studies. The applicant must conduct postmarketing studies, such as field studies, to verify and describe the drug's clinical benefit and to assess its safety when used as indicated when such studies are feasible and ethical. Such postmarketing studies would not be feasible until an exigency arises. When such studies are feasible, the applicant must conduct such studies with due diligence. Applicants must include, as part of their application, a plan or approach to postmarketing study commitments in the event such studies become ethical and feasible.
2. Approval with restrictions to ensure safe use. If FDA concludes that a drug product shown to be effective under this regulation can be safely used only if distribution or use is restricted, FDA will require such postmarketing restrictions as are needed to ensure safe use of the drug product, commensurate with the specific safety concerns presented by the drug product such as:
    - Distribution restricted to certain facilities or health care practitioners with special training or experience;
    - Distribution conditioned on the performance of specified medical procedures, including medical follow-up; and
    - Distribution conditioned on specified record-keeping requirements.
3. Information to be provided to patient recipients. For drug products or specific indications approved under this subpart, applicants must prepare, as part of their proposed labeling, labeling to be provided to patient recipients. The patient labeling must explain that, for ethical or feasibility reasons, the drug's approval was based on efficacy studies conducted in animals alone and must give the drug's indication(s), directions for use (dosage and administration), contraindications, a description of any reasonably foreseeable risks, adverse reactions, anticipated benefits, drug interactions, and any other relevant information required by FDA at the time of approval. The patient labeling must be available with the product to be provided to patients prior to administration or dispensing of the drug product for the use approved under this subpart, if possible.

1.2 Regulatory History of Pneumonic Plague Animal Model Development

A United States Food and Drug Administration/National Institutes of Health (FDA/NIH) Antibiotic Working Group met in 2001-2002 and discussed the development of therapeutic options for pneumonic plague and inhalation anthrax, for the general population. The FDA and NIH selected currently approved antibacterial drugs for a potential treatment indication for pneumonic plague that met the following criteria: had large safety databases, both oral and intravenous formulations were available, adequate production capacity existed, and demonstrated utility in special populations. The National Institutes of Allergy and Infectious Diseases (NIAID) and FDA jointly undertook a program of animal model development, and pharmacokinetic and efficacy studies to address this need. In March 2003, at the request of the FDA, the Division of Microbiology and Infectious Diseases (DMID), NIAID/NIH, established Pre-Investigational New Drug Application (PIND) 64,429 to collect communications, protocols and data for the animal model and testing of antibacterial drugs. The data presented in this package are the results of nearly a decade of effort by NIAID and FDA. In 2003, after the first natural history study (F03-09G), data was presented to FDA and a treatment trigger was selected for subsequent antibacterial drug efficacy studies. The results of two such antibacterial drug efficacy studies were very promising. In 2010, NIAID and Johnson and Johnson (J&J, now Janssen) began discussions to support J&J's supplemental NDA submissions for levofloxacin (NDA 20-634/S-061; NDA 20-635/S-067; NDA 21-721/S-028), and NIAID began planning for a pre-IND submission for the generic drug, ciprofloxacin (PIND 113829). The animal model development program is identical in support of both drugs; antibacterial drug efficacy data are not the subject of this summary.

2 Pneumonic Plague

The goal of this section is to describe the historical and literature-based evidence for understanding the correlation between the AGM model of pneumonic plague and the human clinical disease.

Section 2.1 describes the human clinical disease course of pneumonic plague and the unmet medical need.

Section 2.2 describes the morbidity and mortality associated with human cases of pneumonic plague.

Section 2.3 describes the current options for treatment of pneumonic plague.

2.1 Human Clinical Disease

Plague is caused by an infection with *Yersinia pestis* (*Y. pestis*), a Gram negative, bipolar-staining bacillus and member of the family Enterobacteriaceae. The yersinioses are zoonotic diseases affecting rodents, pigs, birds, and other domestic and wild animals; humans are only

incidental hosts. However, pandemics due to *Y. pestis* have been responsible for large losses of human life and profound changes in society over the past two millennia (Dennis, 2009).

Plague can have several forms. The most common is bubonic plague in which illness typically begins after a 2- to 7-day incubation period following a bite by an infected flea that had been feeding on an infected mammal, often a rat (Dennis, 2009; Inglesby 2000). The bacteria reproduce in regional lymph nodes draining the area of the bite and cause the nodes to swell in size from 1 to 10 cm in length to form a bubo, which is exquisitely tender and firm with the overlying skin being warm and stretched. This is usually accompanied by the sudden onset of fever, chills, weakness, and headache. Some patients develop skin lesions including papules, vesicles, or pustules distal to the bubo that may represent sites of flea bites. Patients may not seek care during the first days of the illness, but when they present clinically, they are typically prostrate and lethargic and may also be restless or agitated. Such patients usually have fever in the range of 38.5ºC to 40.0ºC, but may have higher fever with delirium or, in children, seizures. The pulse rate is typically increased to 110 to 140 beats per minute (bpm), and there may be hypotension due to vasodilatation. The liver and spleen may be palpably enlarged and tender. Without appropriate treatment, shock may develop with a rapidly downhill clinical course culminating in death 2 to 3 days after the onset of symptoms (Dennis, 2009).

Septicemic plague can arise as a result of rapid replication of *Y. pestis* in the blood in early acute cases of bubonic plague or occasionally, it can arise without the development of a bubo (Dennis, 2009; Inglesby, 2000). It is associated with a higher case-fatality ratio than bubonic plague, which may be due, in part, to delays in diagnosis and treatment. Without early intervention, it will produce a systemic inflammatory response that may lead to disseminated intravascular coagulation, bleeding, organ failure, and irreversible shock. Purpuric skin lesions are usually found scattered across the extremities and trunk. At first, they are red but change to dark purple. Blockage of the peripheral vessels in the acral sites (such as the tips of fingers, toes, ears, and nose) can lead to gangrene; hence the term “Black Death” as a pseudonym for plague.

The third, most feared manifestation is pneumonic plague. This can be secondary pneumonia that can arise when *Y. pestis* reaches the lungs by hematogenous spread from a bubo or from the bacteremia of septicemic plague (Dennis, 2009; Inglesby, 2000). It is a malignant pneumonia that is accompanied by sepsis and progresses rapidly to death without prompt medical intervention. In addition, pneumonic plague is highly contagious through respiratory droplet spread, especially through close contact with family and caregivers (Dennis, 2009). Primary pneumonic plague is defined as disease resulting specifically from inhalation of infectious *Y. pestis* respiratory droplets and can be transmitted from human to human without involvement of fleas or animals (Dennis, 2009; Inglesby 2000). Respiratory droplet spread results in inhalation of *Y. pestis* bacteria directly into the lungs, which could be from the cough of another human with pneumonic plague, an infected animal, or perhaps, an intentional release in a bioterrorism event.

Prior to the development of modern antimicrobial agents, pneumonic plague was considered to have an extremely poor prognosis. In most series of cases from the pre-antibiotic era, the mortality rate was essentially 100% (Dennis, 2009). Earlier treatments for plague included carbolic acid, antimony sodium tartrate, Bayer 205 (Germanin), electrargol or collargol (colloidal silver), Eusol (boric acid and chloride of lime), Fonabisit (formaldehyde sodium bisulfate), tincture of iodine alone and mixed with camphor and thymol, intravenous (IV) mercurochrome, perchloride of mercury, and urotropin (hexamethylenetramin). Initial reports gave encouraging results that were not often duplicated. Various serum, bacteriophage, and vaccine therapies were developed and tried with similar results (Chun [a,b], 1936).

Currently available FDA-approved antibacterial drugs for treatment of *Y. pestis* infection include streptomycin, doxycycline, tetracycline, and other antibacterial agents in the tetracycline group. Gentamicin is used to treat plague but is not approved for the indication. Evidence for the efficacy of tetracyclines for the treatment of pneumonic plague is limited. Streptomycin is infrequently used in the US and there are limited supplies (Louisiana Office of Public Health, 2004).

The review of the case reports and summaries of observations from patients with pneumonic plague demonstrates a similarity in the clinical course. Fever, especially high fever, is often the first sign. Cough appears to develop at various times in the course of the illness and may or may not be productive at first, but typically progresses from producing thin, sometimes blood-tinged, sputum to more profuse production that can be substantially bloody. Usually the findings on physical examination of the lungs belie the severity of the pneumonia and prognosis for the patient. Delirium and a staggering walk were observed in many cases. Without early initiation of appropriate antibacterial drug treatment, the case fatality rate is very high.

2.2 Morbidity and Mortality Associated with Pneumonic Plague

This report has been prepared to provide a brief history of plague and a description of clinical manifestations of pneumonic plague infection. Cases are described in terms of clinical signs and symptoms with the time frame of development after infection, as well as the histology and radiology examinations. Clinical case descriptions of untreated pneumonic plague can be found in older literature and provide an overall picture of the clinical course of the untreated *Y. pestis* infection. These case descriptions are summarized below.

2.2.1 1905 Treatise on Plague

In 1897, Dr. Poch initially documented (in the German language) the death of Dr. Manser and his nurse of Bombay, India, and the deaths of Dr. Mueller and a nurse of Vienna, Austria. These accounts were later translated and published in the 1905 Treatise on Plague (Simpson, 1905) and are summarized here.

2.2.1.1 Manser case (Bombay, India)

On the first day of his illness, Dr. Manser had a sudden rigor and felt a fever coming on. Later that day he developed a severe headache, became nauseated and vomited several times. His

limbs ached and he became lethargic. At 2 PM on Day 1, his temperature was 103.4°F, his pulse was 116 bpm, and his respiration rate was 25 breaths per minute (brpm).

On Day 2, after not getting much sleep, he felt worse except for the aching in his limbs. His temperature remained elevated at 103.5 to 104.5°F, his pulse was about 110 bpm, and his respiration rate was approximately 23 brpm throughout the day. The afternoon of Day 2, he felt a pain at the lower part of his left axilla, but no enlarged or tender lymph nodes were discernable.

On Day 3 of his illness, after another restless night, he felt very ill. His temperature had risen to 104.6°F, his pulse was 113 bpm, and his respiration rate was 25 brpm. His tongue was still moist with a little "fur" toward the back, and his previously described symptoms persisted. That night he began to cough up watery sero-mucous fluid that contained minimal amounts of blood. Moist respiratory sounds, reminiscent of early pneumonia, could be heard on auscultation. Microscopic examination of the sputum revealed many bacilli that appeared to be *Y. pestis*, which was confirmed from sputum cultures growing pure colonies of *Y. pestis*.

During Day 3 and Day 4 of his illness, Dr. Manser's condition deteriorated. His temperature remained around 104°F and expectoration became more profuse. Moist rales could be heard throughout his chest. Respiration rate measured at 35, increased further to 45 brpm, and his pulse increased to 120 to 135 bpm. Dr. Manser died early the morning of Day 5.

The evening after the death of Dr. Manser, his nurse became ill and developed symptoms of pneumonia the following day. She rapidly became worse and died on Day 3 of her illness. While her sputum production was not as profuse, she became exhausted much quicker. Examination and culturing of her sputum revealed the presence of *Y. pestis*.

2.2.1.2 Mueller case (Vienna, Austria)

Dr. Mueller appeared to have contracted pneumonic plague from a patient who was a worker in the pathological institute in Vienna, Austria or from one of the nurses who tended to this patient.

One of the patients at the pathological institute in Vienna, Austria died 4 days after presenting to the clinic. The patient's illness was later confirmed bacteriologically to be pneumonic plague. Two days after the death of this patient, one of the nurses that cared for the patient became feverish, and Dr. Mueller volunteered to take care of her. However, that evening (Day 1), Dr. Mueller felt chilled and began to shiver despite the room being well heated. He had a non-productive cough and some aches in his legs, and he felt depressed. Dr. Mueller retired for the night without finishing his dinner; he slept well.

The morning of Day 2 upon visiting the nurse who had become ill, he was reported to be very pale and fatigued. His pulse was 110 bpm, and although his cough was frequent, it was still unproductive. Dr. Mueller took a 3-hour nap, after which, his temperature measured 38.2°C (100.8°F). He then returned to bed. Later that day, he began to expectorate reddish, thin fluid which was found to contain bacilli consistent with *Y. pestis*. His pulse was 120 bpm and his respiration rate was 40 brpm. Coughing became more frequent with production of copious

amounts of reddish sputum. There were no complaints of pain. Fluid, but not bloody, stools were reported the afternoon of Day 2, and in the evening (6 PM), his temperature had reached 40.8°C (105.4°F), and he reported being very thirsty. Digitalis and alcohol were administered. Dr. Mueller refused an injection of plague serum. He had bouts of delirium but was able to sleep.

On the morning of Day 3 of his illness, Dr. Mueller's conjunctivae were reddened. He was delirious and spoke incoherently. He produced large amounts of reddish fluid sputum. No solid food was ingested that day, and a second dose of digitalis and "good quantity" of alcohol were given. In the afternoon of Day 3, he took morphine for chest pain. Four thin, non-bloody stools were produced with pain. At 6 PM that evening, his respiration rate quickened to 59 brpm, and he became cyanotic. Coughing became more frequent with continued production of bloody sputum. His consciousness was dulled. At 10 PM, his body temperature dropped to 37.8°C (100.0°F) and while consciousness seemed to get clearer, restlessness and delirium eventually set in.

At 1 AM on Day 4 of his illness, Dr. Mueller got up and walked with assistance, and returned to bed and slept. Three hours later, his temperature was 38°C (100.4°F) but breathing was difficult and cyanosis had increased. At 4:15 AM, a rattling in his throat was heard and bloody mucus poured from his mouth. He died at 4:30 AM on Day 4.

2.2.2 1924 Los Angeles Epidemic

Other clinical cases of primary inhalation pneumonic plague from the 1924 epidemic in Los Angeles, CA have been described by Bogen (Bogen, 1925). Among the 32 cases, he observed that the primary symptoms of pneumonic plague, in order of frequency, were fever (37.8 to 41.1°C [100.0 to 106.0°F]), expectoration with blood-stained sputum, cough, pain in the chest, headache, generalized pains, vomiting, pain in the back and upper abdomen, malaise, epistaxis, and chilliness without rigor. Primary physical examination findings, in order of frequency, were large, coarse rales in the chest, thickly coated tongue, reddened throat, dyspnea, impairment of percussion noted over the chest, restlessness, prostration, delirium, weak rapid pulse, cyanosis, a systolic murmur, localized adenopathy, conjunctival injection, increased spinal fluid pressure, with signs of meningismus in the child cases, jaundice, and a macular rash. With the exception of 2 patients, all died. Greater than half of these patients received IV or intraperitoneal (IP) injections of mercurochrome, including the 2 survivors.

A summary by Link (Link, 1955) in Chapter VIII of "A History of Plague in the United States of America" provides additional information about this epidemic.

2.2.3 1926 Treatise on Pneumonic Plague

In 1926, Wu Lien-Teh produced "A Treatise on Pneumonic Plague" for the League of Nations. (Wu Lien-Teh, 1926) He was a highly regarded expert in the field because of his extensive experience with pneumonic plague during the epidemics that occurred in Manchuria. Regarding the incubation period for pneumonic plague he wrote, "In the majority of cases, the incubation period is under 5 days; nevertheless,...6 days is frequent enough to deserve serious attention.

Longer periods are very rare.... An incubation of less than 2 days is comparatively rare.... We doubt if such ever occurs."

In Chapter V, Section II: Symptomatology, he provides the following summary of the clinical features of pneumonic plague from personal and reported observations:

> "The onset of the disease is sudden and often marked by rigor. The first stage is characterised by the presence of general signs only; cough is most often still absent; when present, it is usually dry. The prominent symptoms during this period are severe headache, some nausea and vomiting, vertigo and general malaise. Both respiration and pulse show an increased rate; the pulse is early impaired in quality. The temperature, which is but slightly raised at the beginning of the illness, rises steadily during the first stage. Most of the symptoms present during this period appear to be toxic in nature and are best explained by an influence upon the central nervous system. Inasmuch as, during this period no bacteremia is present, it is feasible to assume that some toxemia exists early in the disease.
>
> The beginning of the second stage is manifested by the appearance of cough or - if this is already present - by that of expectoration. The cough is dry and seldom troublesome at first, when continuous may exhaust the patient. The sputum shows at first no characteristic appearance, being mainly frothy. Soon, however, there is an admixture with blood, leading to a uniform bright pink or red hue. Now the sputum may be either thin, sometimes frothy or of more syrup-like consistency; but the degree of viscosity typical for croupous pneumonia is not reached. The quantity of bloody sputum varies greatly from mere streaks of red to ounces of deep-red blood comparable to that seen in hemorrhage in phthisis (tuberculosis).
>
> During the first stage, few if any signs may be detected over the lungs; now symptoms of pneumonia become evident. The comparative insignificance of physical signs in the lungs even in the second stage is in marked contrast to the serious condition of the patient.
>
> The respiration increases in frequency, dyspnea grows more and more marked and the face soon assumes a cyanotic hue. The temperature, which reaches its height at the beginning of the second period, remains high, with little or no matutinal [early in the day] remissions. The pulse is soft, increases in frequency and deteriorates in quality from hour to hour.
>
> The patient becomes dull and assumes an anxious look. Sometimes coma or delirium is present, but usually consciousness is not lost until the very last. Ataxic gait and incoordination of speech are frequent.
>
> Death occurs from heart failure. Sometimes there is a marked stage of agony characterized either by more or less protracted coma and symptoms of lung edema or by restlessness and active delirium. Often death is quite sudden, brought about by some slight exertion. In the Manchurian outbreaks especially, such a sudden painless death was the rule, and corpses were found in all sorts of positions."

In this 1926 treatise, Wu Lien-Teh summarized 41 reports on the duration of illness (refer to Table LVIII in the source reference) (Wu Lien-Teh, 1926). The average duration of illness observed was 1.8 days with the longest duration being 9 days.

2.2.4 Plague: a manual for medical and public health workers

In a text book collaboration by Wu Lien-Teh et al (Chun [b], 1936), the clinical courses of 9 individuals with pneumonic plague during the pre-antibiotic era are described; many of these cases were initially described in his 1926 treatise (Wu Lien-Teh, 1926). These cases are presented here.

2.2.4.1 Case 1

One case of the Manchurian pneumonic plague epidemic of 1921 was that of a female, "Mrs. S", 28 years of age. Mrs. S was the sister-in-law of the first plague patient appearing in Harbin, China on 22 Jan 1921; she was 1 of 4 contacts found living in the same room as this patient (the others were the mother, sister and male friend). It was not expected that Mrs. S would have been in close contact with the patient; however, the whole family was living in one room (12 x 12 square feet).

All contacts were isolated in the hospital quarantine compound, and being the first cases, were examined with special care. On Day 1, Mrs. S was active and loquacious, opposing strongly to the idea of her being deprived of her freedom. On Day 2, she still demanded to be set free, although the other 3 contacts took being quarantined more "philosophically". On Day 3, Mrs. S was quieter and happy when persuaded that, if all went well, on Day 5, the family could go home.

On the afternoon of 26 Jan (Day 5 after contact with the plague patient), Mrs. S felt chilled and unwell. Her pulse was 108 bpm, respiration rate 20 brpm, and body temperature 100.0°F. The next morning, she complained of not having slept well, her pulse increased to 118 bpm, respiration rate to 24 brpm, and body temperature to 101.5°F. Her whole condition seemed to have changed overnight. Her face was pale and drawn, she looked weak and anxious, and her head ached badly. Mrs. S was now separated from the other 3 contacts, and she was unconcerned with conversation.

Toward the evening, symptoms became worse; slight cough and sputum developed, and some pain was felt on the left side of the chest. Her pulse was 120 bpm, respiration rate 30 brpm, and temperature 102°F. Physical signs in her lungs were slight. Her urine was cloudy but no albumin was detected. Within the next 2 hours, blood streaks had appeared in her sputum, which had now increased in quantity. *Pasteurella pestis* (now known as *Y. pestis*) were identified. At this time, the patient's pulse increased to 130 bpm, respiration rate to 36 brpm, and temperature 102.5°F. Water and medicine were requested by the patient to ease her headache and increasing tightness of chest. That evening (10 PM), examination of the peripheral blood showed plague bacilli. General symptoms increased in severity, and the patient became very restless and died in the early morning of the following day, which was Day 6 from the time of contact with her

brother-in-law (the first plague patient). The other three contacts with the first patient remained healthy.

2.2.4.2 Case 2

A second case was that of "Dr. YTM", a male, 24 years of age. Dr. YTM was in charge of district inspection squads from 11 Feb 1921. Duties included visiting suspected plague-ridden houses, bringing back sputum, and making spleen punctures of corpses. He was a thorough worker, conscientious, and always wore proper masks.

On 17 Feb 1921, Dr. YTM felt unwell but continued work as usual. He attended to 30 people that day, but toward the evening, he began to feel unwell. That evening, he had a flushed face, pulse of 120 bpm, respiration rate of 22 brpm, and temperature of 102°F, and complained of severe pain in head and back. He had an injected cornea with photophobia. Dr. YTM was conscious and aware of his fate. Electrargol or collargol (colloidal suspension of silver cations) was injected (10 cc), along with aspirin. He slept poorly that night.

The next day (Day 2), the patient appeared drowsy. His pulse was 124 bpm, respiration rate 30 brpm, and body temperature 102°F. His sputum was blood-stained with pure growth of *Y. pestis* obtained. He was still coherent and rational but had to be roused. Antipest serum (40 cc) and electrargol (10 cc) were injected subcutaneously. Hours later, although delirious, he could recognize people. That evening, his pulse increased to 130 bpm, respiration rate to 36 brpm, and temperature, 103°F; more blood-stained sputum was produced. Another 50 cc of antipest serum was injected with no apparent effect.

On Day 3 of infection, his pulse was weaker at 150 bpm, respiration rate was 40 brpm, and temperature 102.5°F. Tubular breathing and rales were recorded, along with swollen legs and marked delirium. Cough was minimal, but large amounts of sputum were observed with much blood. A third injection (IV) of antipest serum (80 cc) was administered. At midnight on Day 4, Dr. YTM was very delirious with increased sputum with much blood. His urine was scanty and of dark color with trace of albumin. The following morning (Day 5), he was found in the adjoining room dead, lying over the bed with legs touching the ground.

2.2.4.3 Case 3

The third case was that of "Dr. M", a European male, 43 years of age. Of note, Dr. M had lived through the bubonic plague epidemic at Tongshan, China in 1908. Dr. M accompanied Dr. Haffkine on a visit to a plague ward in Russia in Jan 1911. Neither physician wore a protective mask. During this visit, Dr. M examined the chest of a plague patient via percussion and auscultation. Four days later, he complained of headache with fever (temperature 101°F) and fast pulse. Cough and sputum appeared next day. Two doses of antiplague serum (230 cc and 180 cc) were given with no apparent effect. Blood soon appeared in sputum, and when examined, plague bacilli were found in large quantities. The patient rapidly became weakened, as his temperature rose to 103°F, pulse to 140 bpm, and respiration rate to 40 brpm. Dr. M died 6.5 days after the visit to the plague ward.

2.2.4.4 Case 4

In March 1921, "Mrs. L", 17 years of age, lost her husband due to plague. She was then quarantined in a railway wagon. For 6 days she had a normal pulse and temperature, and appeared free of infection. She was released to go home, which was 0.5 miles away from the quarantine wagon. Soon after arriving home, she became ill and was admitted to the hospital. She presented with no cough or sputum. However, the following day, she experienced much cough with bloody sputum, in which *Y. pestis* was confirmed to be present. Her pulse was measured at 130 bpm, respiration rate at 36 brpm, and temperature 103°F. Thirty hours after admission, Mrs. L was pronounced dead. Post-mortem results showed pneumonia in the lungs.

2.2.4.5 Case 5

On 22 Mar 1921, a few days after losing her husband to plague, "Mrs. F", 31 years of age and pregnant with her fourth child, was admitted to the hospital with fever (102°F) and fast pulse (120 bpm) and respiration rate (30 brpm). This was the second or third day of her illness. She experienced much coughing with blood-stained sputum in which plague bacilli were observed. She had a generally weak condition with dark urine without the presence of albumin.

Later the same day, Mrs. F gave birth to a deceased full-term female fetus; the placenta passed without trouble. Mrs. F's general symptoms became worse, and the patient died early the next morning. Post-mortem examination of Mrs. F showed definite signs of plague pneumonia in her lungs. The placenta also showed plague bacilli, and although organs of the fetus appeared normal, plague bacilli were isolated from them.

2.2.4.6 Case 6

"Dr. S", a Russian male, 33 years of age, was in charge of house-to-house inspection in Harbin Railway area from 20 Feb 1921. He was admitted to the hospital on 18 Mar 1921, complaining of fever (temperature 39.6°C [103.3°F]) and pain over the right side of his chest. His pulse was 102 bpm and respiration rate 26 brpm. The patient was conscious and thirsty. On percussion, some dullness below right scapula was recorded, and no rales were heard on auscultation. The following day (Day 2), his pulse was 110 bpm, respiration rate 34 brpm, and temperature 39.7°C (103.5°F). Some delirium was experienced, and on auscultation, large crepitant rales were heard over a dull area. The patient received an injection of camphor with 80 cc serum, along with methylene blue (0.03 g, 6-times daily for 2 days). That evening, his pulse became faster and weaker, and arrhythmia was reported. Cough developed with sputum containing an abundance of plague bacilli.

On Day 3, the patient's temperature ranged from 40.4 to 40.6°C (104.7 to 105.1°F). He complained of a pain below the right collar bone. The patient experienced nausea and vomiting. Dr. S was conscious and could answer questions intelligently, albeit slowly; however, his hearing was impaired and he could not walk properly. His appearance was one of exhaustion, with a reddish-violet face and blue nails. Pulse was measured over 150 bpm and respiration rate greater than 50 brpm. Anti-diphtheria serum was given (5,000 units) along with camphor oil. At 7 PM

that evening, marked cyanosis, irregular breathing, and increased cough with much sputum and blood, were observed. The patient became unconscious and died at 3 AM, the morning of Day 4.

2.2.4.7 Case 7

Case 7 involves 3 refugees from Irkutsk, Russia. All three, looking ill and starved, were admitted to Suspect Block (presumably where suspect cases were held for observation) on 8 Feb 1921. One of the 3 spat blood and died 5 days later (13 Feb) with suspicious signs of plague. A second refugee complained of plague-like symptoms and was put in a plague ward. He died on 17 Feb 1921; however, swabs from his throat and sputum repeatedly failed to show *Y. pestis*.

The third refugee, a 35-year-old male, spent 11 days in plague ward without a mask and without signs or symptoms of infection. He subsequently volunteered as an attendant in the plague ward with inconsistent use of a protective mask. After 3 weeks, he showed signs of plague with fever, fast pulse, and respiration rate of 30 brpm. Soon after, he produced bloody sputum, in which plague bacilli were identified. There were also clinical signs in his lungs. Eusol (120 cc IV) was injected. The patient quickly became unconscious and breathing was “stertorous”. Within 24 hours of symptoms first appearing, the patient died. Therefore, former contact with plague patients did not produce immunity.

2.2.4.8 Case 8

On 15 Mar 1921, a male, 32 years of age was admitted for care, presenting with typical plague symptoms (fever, fast pulse and quickened respiration). His sputum, initially scanty, produced *Y. pestis*. The patient retained his appetite and remained stable for days. His sputum was examined every 2 days and resulted in small numbers of *Y. pestis*. Subsequent specimens were darker and mucopurulent; *Y. pestis* was always present. The patient was secured in a special ward, as his recovery appeared hopeful.

On 21 Mar (5 days after admission), the patient received 60 cc serum. Fever remained high, ranging from 102 to 102.5°F, pulse increased from 114 to 120 bpm, and respiration rate from 24 to 36 brpm. Some rales in both lungs were documented, as was albumin in his urine. Over the next 2 days, his condition worsened; pulse quickened to 140 bpm and respiration rate was nearly 50 brpm. The patient died the next day, 9 days after admission into the plague ward. This was the longest survival time on record following *Y. pestis* infection in the pre-antibiotic era. Post-mortem examination showed marked pleuritis and pneumonia; plague was confirmed.

2.2.4.9 Case 9

The ninth case is that of a 21-year-old male rat catcher who arrived in Lungyen (Fukien Province, Manchuria) on 18 Jul 1935 (Chun [a], 1936). For 10 days he trapped rats and searched for plague cases without success. Therefore, *Y. pestis* infection seemed unlikely. In addition, this man participated in the fumigation of 4 houses with sulphur gas. Collection and transportation of dead rats was always performed with the proper protection.

On July 28, 10 days after his arrival to this region, he complained of pain in the right side and in the back of the chest with a noted slight fever. The following day (Day 2), his pulse was 92 bpm

and temperature 102°F. The patient was not feeling well and suffering from severe backache, but without cough or headache. On the morning of Day 3, cough and bloody, frothy sputum appeared. Microscopic examination of sputum smears showed many plague bacilli – some in chain formation. Toward evening, both cough and sputum increased with the sputum becoming liquid. Tightness and pain in his chest had become worse, and severe headache was reported.

On Day 4, the patient became delirious. Expectoration of liquid blood-stained sputum continued, and his pulse rose (110 to 120 bpm) and temperature reached as high as 105°F. The patient died the morning of Day 5.

2.2.4.10 Summary

Based on these cases, Chun provided a description of a typical clinical course for a patient with pneumonic plague which is described below (Chun [a], 1936).

> "From the onset of illness, the patient suffering from primary plague pneumonia will show signs of serious infection and feel suddenly unwell. He/she may be prostrated with mental confusion, severe headache, pain in the back and pain at the site of the bubo (if present), chills or rigor, a coated tongue, injected conjunctiva, and loss of muscle coordination for walking to the point of staggering. There may be vomiting and/or diarrhea (sometimes bloody). Within 24 to 36 hours, body temperature will be raised to 40.0°C (104.0°F) or higher and pulse, quickened to 110 to 130 bpm. The patient may have an anxious expression. The face will often be flushed and bloated and with time assume a dark, dusky hue. The mucous membranes of the mouth and the fauces typically will be congested and cyanotic. Usually, the spleen will not be palpable and lymphatic glands, not enlarged. Leukocytosis may be absent.
>
> In general, the patient will complain of pain and a restricted feeling in the chest that is not severe. Cough and dyspnea develop. The cough is usually easy and not painful. At first, the cough is dry and sputum is scanty, but subsequently, the sputum becomes more abundant and blood-tinged, and later, it becomes thinner and frothy with a bright red color. At this point, the sputum is highly contagious as it contains almost a pure culture of *Y. pestis*.
>
> The physical signs in the lungs are often slight, even in the advanced stages. In some cases, local areas of dullness on percussion may be observed. Rales may be present on auscultation but are not frequently heard except just before death when numerous rales can be heard due to pulmonary edema. A dry pleuritic rub may be heard at the side of the chest. The heart is usually slightly dilated on the right side; heart sounds are fast and become more feeble. The second pulmonary sound may be accentuated early on but this character is soon lost.
>
> In the later stages of the illness, the respirations become increased with marked dyspnea and gasping for breath several hours before death. Cyanosis is common and ecchymoses appear in different parts of the body. The pulse becomes more rapid, soft and feeble until it cannot be felt. Body temperature may decline to or below normal near the end of life. The patient is usually delirious (even may be inappropriately cheerful) or comatose. Immediately before death there may be profuse hemoptysis with bloody froth seen at nostrils. Death results from cardiac paralysis and exhaustion. Patients sometimes succumb after slight physical exertion, such as sitting up in bed or being moved. Death occurs 2 to 3 (rarely 4) days after infection."

2.2.5 Radiographic Findings in Untreated Pneumonic Plague

Alsofrom et al (Alsofrom, 1985) reported radiographic findings of 9 patients with probable (n = 5) and confirmed (n = 4) secondary pneumonic plague, in addition to findings from 28 patients with bubonic plague and 5 patients with septicemic plague. This summary focuses on the pneumonic plague cases.

All 9 pneumonic plague patients revealed alveolar pulmonary infiltrates, 8 of which were bilateral. In general, the infiltrates were non-segmental and within the alveoli with a propensity for the lower lobes. Mediastinal or hilar adenopathy, as well as pleural effusions, were observed in both bubonic and pneumonic plague patients. Five (55.6%; 5/9) pneumonic plague patients had cervical adenopathy and one had supraclavicular adenopathy; in contrast, only 2 of the 27 (7.4%) bubonic plague patients with a radiography assessment had cervical involvement.

A total of 3 (33.3%; 3/9) pneumonic plague patients died. Treatment received by these patients was not reported; therefore, it is difficult to draw conclusions from this report.

2.2.6 Summary of Untreated Clinical Course of Pneumonic Plague

In reviewing these case reports and summaries of observations from patients with pneumonic plague, the overall similarity in the clinical course is apparent, independent of presentation. Fever, especially high fever, is often the first sign. Cough appears to develop at various times in the course of the illness and may or may not be productive at first, but typically progresses from producing thin, sometimes blood-tinged, sputum to more profuse production that can be substantially bloody. Usually the findings on physical examination of the lungs belie the severity of the pneumonia and prognosis for the patient. Delirium and a staggering walk were observed in many cases. Without early initiation of appropriate antibacterial drug treatment, the case fatality rate is very high.

2.3 Antibacterial Drug Treatment of Pneumonic Plague Described in Medical Literature

Several early generation antibacterial drugs have been used in the treatment of pneumonic plague and reported in the literature. Several cases of pneumonic plague where antibacterial drug treatment was administered are described below.

2.3.1 Treatment with Sulfadiazine and Penicillin

A report by Munter describes a laboratory worker infected with *Y. pestis* and successfully treated with sulfadiazine (Munter, 1945).

The laboratory worker “Dr. JH”, who worked with a virulent strain of *Y. pestis* at the Plague Investigation Station of the US Public Health Service in San Francisco, CA became ill on 31 May 1944. Approximately, 1 year prior, he had received 5 injections of plague vaccine over 6 months due to the risk of infection through his research. This vaccine was prepared from virulent *Y. pestis* which had been chemically killed.

The initial onset of malaise, chills and hacking cough was accompanied by fever (104°F) and blood-tinged sputum. He was admitted to the hospital on Day 2 of onset, complaining of malaise, pain in the chest (low down on the right side), and a frequent, non-productive cough. He was aware but confused and disoriented. His temperature was 104°F, pulse was 100 bpm, respiratory rate was 26 brpm, and his blood pressure was 110/70 mm Hg. His throat was reddened, and no cervical, axillary, or inguinal adenopathy was present. There was an area of impaired resonance at the back base of the right lung. Breathing was normal except for fine scattered rales, and there was a faint systolic murmur at the apex of the heart. White blood cell count was 18,000 with 88% neutrophils and 12% lymphocytes. Examination of his sputum within 24 hours of admission revealed large numbers of bacteria compatible with *Y. pestis*, which were subsequently confirmed.

Treatment with sulfadiazine was started immediately beginning with 12 g orally (per os [PO]) within the initial 20 hours and 1 g every 4 hours thereafter. After 24 hours, blood levels of sulfadiazine were 4 mg/mL; therefore, 2.5 g of sodium sulfadiazine was administered IV, and the oral dose was increased to 2 g every 6 hours. On Day 4 following onset of symptoms, penicillin (100,000 units/day) was started and given every 24 hours for the next 3 days. There was no evidence that penicillin altered the course of infection.

Over the first 4 days, his temperature ranged from 102.2 to 106.8°F. Coughing continued but was not violent and produced small amounts of sputum. During the first 6 days, the patient experienced delirium and wildly irrational behavior for which he was given morphine and scopolamine. On Day 6, body temperature decreased with signs of improvement. His cough was milder and less productive; at this time, no plague bacilli were cultured from the sputum. On Day 8, his temperature returned to normal and stabilized. On Day 26, the patient was discharged for a long convalescence. Clearing of infiltration was observed via chest x-ray 6 weeks later. Recovery appeared to be complete and no secondary cases developed.

This was the first reported case of primary pneumonic plague treated by sulfadiazine with recovery. However, recovery cannot be attributed solely to administration of sulfadiazine. As noted above, Dr. JH had received plague vaccine approximately 1 year prior to his illness. It is unclear if this vaccine provided protection. The initiation of treatment within 26 hours of symptoms onset may have been a critical factor in successful recovery of this patient.

In addition to the summary above, Tieh, et al. reported on an outbreak of primary pneumonic plague in 1946 (Tieh, 1948), where 5 of the 39 patients, not previously vaccinated, were given sulfadiazine. Drug treatment was effective in 3 of the 5 patients, and the 2 deaths were presumably due to not receiving treatment early enough.

2.3.2 Treatment with Sulfadiazine, Sulfamerzine, and Streptomycin

A bacteriologist, 33 years of age, working at the National Institutes of Health, Nanking, China, became ill from an accidental laboratory infection with *Y. pestis* (Huang, 1948). Due to the relevance of his work, the man had been vaccinated with 2 injections of plague vaccine from

Haffkine Institute in Nov 1942 and a single injection of a plague vaccine prepared by the National Epidemic Prevention Bureau, China, in Dec 1943.

On the evening of 11 Feb 1947, the man began to have generalized malaise, aches and fever, followed by headache and chills the next morning. Two days later (Day 3), he developed a non-productive cough. At this time, his wife, a nurse, gave him 2 g of sulfadiazine. A physician was consulted. The patient was observed to have respiratory distress and dullness of the right lower lobe with diminished breath sounds but no rales. Lobar pneumonia of the right lower lobe was suspected, and an additional 2 g of sulfadiazine was administered that evening followed by 1 g every 4 hours. Approximately 3 AM on 14 Feb (Day 4), he began to cough up thin, bloody sputum. *Y. pestis* was suspected from microscopic examination of the sputum, which was confirmed by culture and animal inoculation. The patient was moved to a temporary isolation quarter where he stayed during the course of his illness.

Sulfadiazine was started approximately 48 hours and streptomycin, approximately 72 hours after onset of illness. The patient received a total of 97 g sulfadiazine between 13 Feb (Day 3) and 03 Mar (Day 21), when it was discontinued due to low white blood cell count. Sulfamerzine was initiated, and a total of 104 g were given between 04 Mar (Day 22) and 03 Apr (Day 52). Streptomycin intramuscular (IM) injections were started on 14 Feb (Day 4) at 100,000 units every 3 hours. The dosage was increased to 2 to 3 million units per day when supplies of the drug improved. The patient received a total of 21,440,000 units between 14 Feb and 03 Mar. Gram-negative bacteria reappeared in his sputum, and streptomycin was restarted on 17 Mar at 3 million units per day; a total of 18,150,000 units were administered over the next 7 days. Penicillin (1 million units) was also given from 22 Feb through Mar 4 with the goal of preventing a secondary infection with Gram-positive diplococci.

For the first 2 weeks of illness, the patient was critically ill - mentally clear but occasionally drowsy and unreasonable. He complained of right-sided chest pain. Respirations were labored with occasional cyanosis, and he continually produced bloody sputum. On Day 8, chest examination revealed dullness and diminished breath in the right lower lobe; rales were also present. Fever subsided on Mar 3 (Day 21), and within another week, coughing lessened and became less productive. Chest findings began to improve slowly in early April.

To complicate matters, the patient contracted malaria from a blood transfusion given in the first 2 weeks of illness; quinine was given and he promptly recovered. The patient also developed jaundice for 10 days, starting 21 Apr. The condition resolved with a proper diet and vitamin supplements. The patient was released from isolation on 07 May (Day 86).

Sputum cultures were obtained throughout the course of illness. A pure culture of *Y. pestis* was obtained on Day 4; this strain was sensitive to both streptomycin and sulfadiazine. Slight synergy of the drugs in combination was observed. Bacilli isolated 26 Feb through 16 Mar were found to be avirulent in animal tests, and no Gram-negative bacilli were found after 16 Mar. The

organism isolated on 26 Feb was resistant to streptomycin, with no inhibition of growth regardless of drug concentration, and sensitive to sulfadiazine.

A total of 49 people who had lived on the same floor as the plague patient prior to his isolation received sulfadiazine (3 g per day for 1 week). In addition, 15 close contacts received sulfadiazine (6 g per day for 1 week) started after the confirmation of *Y. pestis* infection. None of the contacts contracted the disease.

2.3.3 Treatment with Tetracycline and Streptomycin

In 1959, a laboratory-acquired case of pneumonic plague occurred at the US Army Medical Unit, Walter Reed Army Medical Center, Fort Detrick, Maryland (Burmeister, 1962). A male chemist, 22 years of age, analyzed cultures of *Y. pestis* for nucleic acid content was possibly exposed to the organism on 27 Aug 1959. Of relevance, he had been vaccinated against plague in March of the same year with a booster on 10 Aug.

At 5 PM on 01 Sep (Day 1), this patient was admitted to the hospital complaining of chills and fever for the past 9 hours, non-productive cough with pleuritic chest pain for 5 hours, and severe backache lower down with stiffness of the neck for 4 hours. On admission, he appeared to be acutely ill holding his back and neck stiff. His deep, dry, coarse cough was outwardly painful. Body temperature was measured at 103°F, pulse was regular (96 bpm), and blood pressure was 100/60 mm Hg. The patient's throat was slightly reddened. A dullness over the left upper lung was observed, and breath sounds were decreased over the entire left lung. Laboratory tests revealed a mild leukocytosis with minimal left shift and an elevated C-reactive protein. Chest x-ray at admission showed a small, ill-defined infiltrate in the left intraclavicular area.

Thirteen hours after the onset of symptoms (Day 2), the patient was given tetracycline (3 g PO). At this time, body temperature had risen to 105.2°F, pulse to 110 bpm, respiratory rate to 30 brpm, and blood pressure was 95/60 mm Hg. He remained alert and cooperative. Late that night, his cough became more coarse and productive of blood-streaked sputum. Moist rales developed in the left intraclavicular area, and a friction rub could be heard. Chest x-ray revealed increased patchy and linear infiltrate in the left upper lobe. His leukocyte count had risen. Examination of his sputum confirmed pneumonic plague, and the patient was isolated.

Shortly after midnight (Day 3 of symptoms), tetracycline (1 g IV) was administered due to oral intolerance. At 3 AM, his temperature was 105°F, pulse was 120 bpm, and respiratory rate was 40 brpm. He was flushed and hazy but remained alert and oriented. Cough continued unassociated with pain, and minimal amounts of sputum were produced at this time. Two hours after the start of IV tetracycline, streptomycin (1 g IM every 6 hours) was initiated, and the rate of tetracycline was increased to 1 g IV every 12 hours. By mid-morning on Day 3, body temperature had decreased to 102°F respiratory rate to 28 brpm; however, the cough persisted. Later temperature was measured at 100.6°F, and the patient was able to tolerate oral fluids.

The patient remained afebrile on Day 4; however, in the afternoon of the 5th day, body temperature rose (102°F). Despite this, the patient appeared to improve overall. Tetracycline

(750 mg PO every 6 hours) was reinstituted. A sputum sample obtained that evening revealed only an occasional intracellular plague bacillus. Temperature returned to normal the following morning, and the patient remained afebrile thereafter.

Streptomycin was discontinued on Day 7; a total of 19 g had been received. A total of 30 g of tetracycline was administered over 10 days. He continued to improve and was discharged 11 days after onset of symptoms, when his chest x-ray revealed complete clearing. *Y. pestis* was isolated by culture and guinea pig inoculation from sputum specimens obtained at admission and after 24 hours; no subsequent blood specimens were positive.

Hospital personnel were given sulfadiazine (3 g per day) for 6 days. There were no secondary cases.

2.3.4 Treatment with Chloramphenicol and Doxycycline

In 2004, a cluster of 4 cases of pneumonic plague occurred in Uganda - 2 concurrent index patient/care-giver pairs (Begier, 2006). Of these, 3 patients received antibacterial drugs. One patient received chloramphenicol IV for 10 days and PO for 8 additional days; this was the lone survivor.

The first index patient, a 22-year-old woman, had symptoms of headache, fever, and chills for several days, followed by lymphadenopathy on Day 3. Cough was first noted on Day 5 becoming productive with bloody sputum on Day 7. Chloroquine was initiated for malaria on Day 6 for 3 days. Her productive and bloody cough increased in severity and on Day 9, she died. This patient's mother, a 40-year-old female, became ill 5 days after her daughter's death. On Day 1, she reported headache, fever, chills, weakness, chest pain, and a productive cough. Penicillin (IM, 6-times over 36 hours) was administered for presumptive severe pneumonia. She died on Day 3 of her illness.

A second index patient, a male, 25 years of age, complained of headache, fever, and chills. The man received anti-malaria treatment and penicillin (IM) for presumptive severe pneumonia. On Day 5 of his illness, his cough was productive with bloody sputum, and he died on Day 6. Five days after the man's death, his sister and primary caregiver, 30 years of age, developed dyspnea and elevated temperature 39.3°C (102.7°F) with respiratory rate at 56 brpm. Assistance was required to walk. Examination of her chest revealed bilateral coarse crepitations. Twenty-nine hours after onset of illness, chloramphenicol (2 g IV bolus) and doxycycline (100 mg PO) were administered. Upon admission to the hospital, chloramphenicol (1 g IV) was administered 1.75 hours after the initial dose (the staff was unaware of the previous dose). Chloramphenicol (1 g IV every 6 hours) was continued for 48 hours, then at a reduced dose (750 mg every 6 hours) for a total of 10 days of IV treatment. She was discharged on Day 10 and given chloramphenicol (750 mg PO every 6 hours) for an additional 8 days. Three weeks later she had fully recovered.

Frontal chest radiographs taken on Days 2, 3, and 18 of illness revealed bilateral airspace disease predominantly in the lower lobes and bilateral pleural effusions without evidence of hilar or

mediastinal lymphadenopathy. These findings were consistent with multilobar pneumonia that improved over that time.

Y. pestis was identified from the patient's sputum by polymerase chain reaction and a direct fluorescent-antibody microscopy using *Y. pestis*-specific antibody. Bacterial cultures of her sputum, collected shortly after treatment administration and suboptimally stored and transported, did not yield *Y. pestis*.

All identified close contacts of the caregivers received cotrimoxazole (960 mg PO twice per day) for 3 days.

2.3.5 Summary of Treated Clinical Course of Pneumonic Plague in Medical Literature

The initial symptoms of untreated pneumonic plague are as described in Section 2.2. In the cases described for which *Y. pestis* infection was confirmed in the first 24-48 hours and antibacterial drugs started immediately thereafter, symptoms seem to have been alleviated over the following one to two weeks.

3 The African Green Monkey (AGM) Model of Pneumonic Plague

The goal of this section is to describe the data from studies conducted to describe the clinical course of disease in untreated African Green Monkeys.

Animal model development studies were performed to assess the median lethal dose (LD_{50}) of aerosolized *Y. pestis* in African Green Monkeys (AGM) and to characterize the natural history of disease progression in the AGM. Studies at all sites relied on the LD_{50} determined in a single study, funded by the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). Several laboratories conducted studies related to the development of the animal model; these studies were all funded through the National Institute of Allergy and Infectious Diseases (NIAID) at the National Institutes of Health (NIH). The studies conducted in the development of the non-human primate animal model for aerosol exposure to *Y. pestis* were not intended to be Good Laboratory Practice (GLP) compliant however good documentation practices and good scientific practices were used. Because these studies were the first studies conducted in this model at these test sites, many Standard Operating Procedures (SOPs) specific to the animal model activities were under development. The studies provided the mechanism by which SOPs were developed and refined for use in later GLP-compliant efficacy studies.

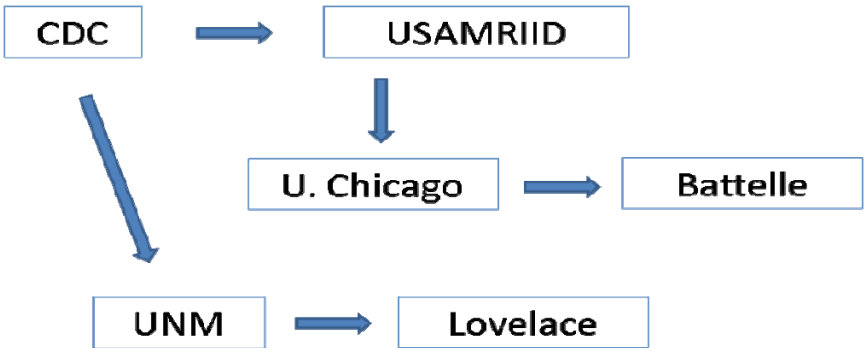
Three laboratories conducted studies to document the natural history of the pathogenesis of *Y. pestis* infection following aerosol exposure: USAMRIID (Frederick, Maryland, USA), the Lovelace Respiratory Research Institute (LRRI) (Albuquerque, New Mexico, USA), and the Battelle Biomedical Research Center (BBRC) (Columbus, Ohio, USA). While multiple laboratories performed studies for NIAID, studies were harmonized only to a limited extent. Variables were harmonized on a global level, but not managed on a local level. All studies used the same species, challenge strain, challenge route, challenge dose and to the extent possible, same parameters measured within studies. These studies were performed as independent, stand-

alone studies and were not prospectively set up to collect exactly the same data using exactly the same methods or assays as would be done if this was one multicenter study. The data from all three laboratories support the animal model and are summarized here, with particular differences highlighted.

The species of non-human primates chosen was the AGM. The AGM is an Old-World non-human primate which is relatively disease free. The AGM is used in a wide variety of research areas and is widely used in biomedical research as an alternative to rhesus macaques. All animals were non-captive bred from the island of St. Kitts, though they may have been acquired from different commercial suppliers (Three Springs Scientific, Inc.; Alpha Genesis). Since the animals are non-captive bred, their age and prior health status are not controlled. All animals were adults, weighing approximately 3 to 7 kg and all were experimentally naïve. Given the source of the animals, they are as comparable from study to study as can realistically be expected and more reflective of variability in the human population than a captive-bred colony. AGM are uniformly and predictably sensitive to *Y. pestis* by subcutaneous injection (Meyer, 1974) and have been used exclusively at USAMRIID since 1993.

The challenge material in all studies was *Y. pestis*, isolated originally from a single fatal human case identified in Colorado in 1992 (Doll, 1994; Opulski, 1992) and designated as CO92 (Friedlander, 1995; Davis, 1996). While all studies used CO92, there was no attempt to control passage history or provenance, though all came from the original source, obtained by the Centers for Disease Control and Prevention (CDC), as represented diagrammatically in Figure 1. Methods for growing the stock material for challenge are described in each study summary and while not identical, are not known to contribute significantly to inter-study variability. These methods have not been formally optimized for this model, nor is a formal optimization planned.

Figure 1 Provenance of *Y. pestis* CO92



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.

All animals were exposed by the aerosol route, which produces the most lethal disease among typical infections (bubonic, septicemic and pneumonic) and is considered to be the most likely route used in a terrorist event. The aerosol challenge technology used among the three

laboratories is very similar though not exact replicates. All three sites use Collison nebulizers and head-only aerosol chambers. The duration of the challenge is based on the animal's inhaled minute volume, measured just prior to (USAMRIID) or during (LRRI, BBRC) challenge. All laboratories measured the challenge dose generated in the aerosol by collection in all-glass impingers. Subsequent microbiological verification of the challenge dose was performed for each animal and these data are presented in each study report.

The target challenge dose selected for the natural history studies and the antibacterial drug efficacy studies was 100 LD_{50} equivalents, which is roughly equivalent to 2.5 LD_{99}. Historically, all animals challenged at USAMRIID with 50 to 200 LD_{50} equivalents by aerosol exposure succumbed to disease. Therefore 100 $LD_{50} \pm 50$ LD_{50} equivalents was the chosen target challenge dose for all the natural history studies and the antibacterial drug efficacy study.

To the extent possible, all animals in the natural history and antibacterial drug efficacy studies were monitored in a similar fashion. The primary measures of interest were mortality, body temperature and bacteremia, with additional secondary measures common to all studies (pathology, hematology and clinical chemistry) and measures included in specific protocols (chest radiographs, coagulation indices, etc.). All animals underwent surgery to implant telemetric monitoring devices to remotely capture physiological parameters such as body temperature, and heart rate; the specific parameters monitored depended upon the device used. Bacteremia was also assessed daily, though the assays used to determine bacteremia were slightly different, such as qualitative and quantitative measures using a variety of growth media. Hematology, clinical chemistry, and chest radiographic assessments were driven by the instruments available in the laboratories. Coagulation indices were included where possible but were anticipated to be indicative of late events in the pathogenesis of disease.

This summary provides an overview of the data from the natural history study reports. In addition, NIAID performed independent reviews of the pathology and radiology findings in these studies to achieve consistent terminology; these reviews are presented in Appendix A and Appendix B, respectively. Conclusions from the independent reviews are identified as such and all other conclusions use data directly from the study reports.

3.1 Determination of the Median Lethal Dose of *Yersinia pestis* in African Green Monkeys

The median lethal dose (LD_{50}) of *Y. pestis* delivered via aerosol was determined in a single exposure study in 16 African Green Monkeys (USAMRIID D93-16). This was conducted under a research protocol and was not intended to be conducted in compliance with GLP requirements.

Sixteen animals were assigned to one of four dose groups (four animals per group), receiving log dilutions of CFU of *Y. pestis* CO92 by head-only aerosol exposure. In-life parameters recorded were daily cage-side clinical observations. Microbiological analyses were limited to determination of individual animal challenge doses. Nine animals in the three highest dose groups succumbed to pneumonic plague. A complete gross necropsy was performed on all

animals that succumbed and all findings were recorded. A complete set of tissues were collected and fixed in 10% neutral buffered formalin for histopathology. In addition, tissue impression smears of lung, spleen, and blood-tinged fluid at the nares were collected for bacteriology. Portions of spleen and lung were collected for electron microscopic evaluation.

The cause of death of all nine of the animals that died on study was primary pneumonic plague; eight of the nine animals had secondary bacteremia characterized as moderate and consistent with *Y. pestis* infection. The ninth animal had minimal bacteremia consistent with *Y. pestis* infection and so bacteremia was not listed as the secondary cause of death. Time to death ranged from 4 to 9 days, with an average of 6.3 days. All nine animals that died on study had pneumonia in both lungs, with the majority (5 of 9) being characterized as marked in both lungs and the rest being marked in one lung and either moderate or minimal in the other lung.

All nine animals had serosanguinous exudates, in both pleural cavities for seven animals and one pleural cavity for the remaining two animals. The majority of exudates (5 of 9) were characterized as moderate, with three animals being characterized as marked and one as minimal.

All nine animals had microscopic findings in spleen (minimal to moderate), liver (minimal to moderate) and bone marrow (minimal to marked) with only a few corresponding gross observations. Lymph nodes had mild to moderate findings in eight of nine animals. Meningitis was observed in three animals, two included gross findings characterized microscopically as mild, and the third was not noted at the time of necropsy and was considered minimal microscopically. Pathology results from these nine animals were published along with 14 other unvaccinated control animals (Davis, 1996).

Statistical analysis of group data resulted in a determination of LD_{50} for use in subsequent studies in this animal model.

3.2 Natural History of Pneumonic Plague in African Green Monkeys

The first detailed natural history study of pneumonic plague in AGM was performed at USAMRIID in June, 2003 (F03-09G). Results from this study allowed the subsequent testing of antibacterial drugs in a treatment setting at USAMRIID. Subsequent efforts required transfer of the pneumonic plague model to additional laboratories. Additional natural history studies were carried out at LRRI (FY06-126 in April 2007) and BBRC (617-G607610 in July 2007, and 875-G607610 in January 2009). These were the first studies at each of these laboratories to assess disease progression in the AGM exposed to inhaled *Y. pestis*; therefore they were not intended to be conducted in compliance with GLP requirements. Good documentation practices and good scientific practices were followed.

3.2.1 Natural History Study of Pneumonic Plague in the African Green Monkey (Study No.F03-09G-Phase-1)

The objective of this study (F03-09G) was to evaluate clinical signs of pneumonic plague disease after aerosol challenge of AGM, such that clinical signs could be used in future studies

conducted in the animal model to assess the treatment efficacy of therapeutic agents. This study was conducted in two phases, and only the first phase is summarized here, as the second phase tested an antibacterial drug. Six healthy, experimentally naïve, adult AGM (3 male, 3 female) weighing 3.5 to 6.0 kg were placed on study. Prior to challenge, the animals had telemetry devices implanted, as well as vascular access ports for blood sampling. Eighty hours of baseline telemetry data were collected, with average readings every thirty minutes.

The challenge material was prepared by inoculating Tryptose blood agar base (TBAB) slants with *Y. pestis* strain CO92 and incubating for two days at 26 to 30°C. On the day of challenge, the slant cultures were suspended in Heart Infusion Broth (HIB), pooled, vortexed and the concentration was determined by optical density reading at 620 nm. The material was diluted in HIB to the target concentration.

On the day of challenge, AGM were anesthetized and minute volume was measured by whole-body plethysmography. Animals were then immediately exposed to the *Y. pestis* aerosol in a head-only chamber, with a target dose of 100 $\pm$ 50 LD_{50} equivalents. The duration of each aerosol exposure is directly related to the animal's minute volume to achieve the target challenge dose. Each animal's actual challenge dose was verified retrospectively by collecting the aerosol during the challenge in an all glass impinger and quantifying organisms by plating dilutions.

Post-challenge, blood was drawn each day for microbiology, hematology and clinical chemistry. Clinical signs were documented twice daily and telemetry recording resumed every 30 minutes. When clinical signs such as fever ($\geq$1.5°C above baseline for 2 hours) or increased respiration (assessed manually) indicated disease, animals were anesthetized for a chest radiograph and blood drawn for microbiology, hematology and clinical chemistry; timing was 80 or 83 hours post-challenge. Clinical signs were also recorded more frequently for animals showing signs of disease (euthanasia scores); four areas were scored (activity, behavior, stimuli response and breathing; 5 points each) with a possible score of 20 for a normal animal and a score $\leq$5 triggering euthanasia. Microbiology during the post-challenge period was measured qualitatively by inoculating a BacTec™ PedsPlus™ culture bottle with 0.5 mL of blood and placing it in the BacTec™ instrument. Animals that met the pre-specified euthanasia criteria were anesthetized for a final radiograph prior to euthanasia. All animals that were found dead or euthanized were necropsied and gross and microscopic observations were recorded. Blood was drawn at euthanasia/necropsy for quantitative microbiology, by plating dilutions on blood agar.

Animals received between 9 and 57 LD_{50} equivalents (Table 1) with a mean dose of 25 ± 17 LD_{50} equivalents; four of the animals succumbed to disease, and those animals received more than 20 LD_{50} equivalents. The two animals that survived the challenge received 9 or 12 LD_{50} equivalents and never showed clinical signs or became bacteremic. These two surviving animals developed titers to the F1 and V antigens of *Y. pestis* and were therefore considered to have been exposed to the bacteria. One animal (V113) was qualitatively bacteremic at 48 hours, and all four animals that became bacteremic were positive by 72 hours and remained positive until death. At approximately 72 hours, animals started to demonstrate a fever (body temperature

increase of ≥1.5°C over baseline) by telemetry. Fever was the first clinical sign of disease and retrospectively correlated very well with bacteremia. Once fever was noted, body temperature did not return to normal until falling rapidly just prior to death/euthanasia. The two survivors, while unexpected, reinforced the correlation between fever and disease, as those animals maintained a diurnal temperature pattern without a fever and never became bacteremic. Two animals showing signs of severe disease were euthanized while two additional animals succumbed after anesthesia though before euthanasia could be completed (see table note, Table 1). Respiratory rates increased only slightly at the time of fever and more dramatically as animals approached death/euthanasia. Clinical scores decreased only slightly at the time of fever in three animals (V627, V514, V113) and more dramatically in all animals as they approached death/euthanasia. Clinical scores and respiratory rates were normal and relatively unchanged in survivors over the course of the study. The number of animals that succumbed to disease in this study is small, and therefore, there were no significant differences between the sexes on time to death or terminal bacteremia levels, nor on development of disease. There were different profiles of white blood cells between males and females that developed disease, with males showing a terminal increase in their total white blood cells and females showing a terminal decrease. The significance of this observation is not understood and requires further exploration.

A complete gross necropsy was performed on all animals that succumbed to disease and all findings were recorded. The following tissues were collected at necropsy and fixed in 10% neutral buffered formalin: nares and lip, skin with mammary gland, submandibular salivary gland, mandibular lymph node, axillary lymph node, inguinal lymph node, mesenteric lymph node, brachial plexus, quadriceps muscle with sciatic nerve, heart, stomach (fundus and pyloris), duodenum, jejunum, ileum, cecum, proximal and distal colon, liver, gallbladder, spleen, pancreas, kidneys, adrenal glands, urinary bladder, prostate or uterus, gonads, eyes, pituitary gland, brain, femoral bone marrow, tongue, larynx, thyroid, parathyroid glands, trachea, esophagus, mediastinum, and lungs. Gross and microscopic necropsy findings for the four animals that succumbed are summarized in Table 2 and Table 3.

Table 1 F03-09G-Phase-1 Natural History: Challenge Dose, Survival, Fever, Bacteremia Observations

Animal ID	Sex	Challenge Dose, LD_{50} equivalents	1st Bacteremia, hours	Time to Fever, hours[a]	Respiratory Rate at 99 hours	Terminal Respiratory Rate	Outcome[b]	Time to Death, hours	Lowest Clinical Score	Terminal Bacteremia, CFU/mL
V627	M	57	72	72	80	140	EU	111.5	9	8×10^7
V514	F	30	72	72	88	146	EU	111	5	3×10^6
V569	M	23	72	67.5	128	128	D[c]	99.5	12	9×10^8
V113	F	21	48	74	72	168	D[c]	125	5	1×10^8
V605	M	9	--	--	36	--	S	--	19	--
V521	F	12	--	--	32	--	S	--	17	--

[a] Fever was defined as ≥ 1.5°C body temperature increase over baseline. Body temperature was monitored every 30 minutes via implanted telemetry devices.

[b] EU = euthanized; D = died; S = survived

[c] Animal met euthanasia criteria and died before it could be euthanized; see p. 197 or p. 201 of NIAID-Yp-NatHis-Path-2011

Table 2 F03-09G-Phase-1 Natural History: Prominent Gross Lesions

Animal ID	Sex	Red/purple foci in lungs	Fluid in trachea or bronchi	Mediastinal edema	Pleural effusion	Tracheobronchial lymphadenopathy
V627	M	+++[a]	+++	+/++	15-20 mL	++
V514	F	++	+++	+	neg	+
V569	M	++/+++	+++	++	30 mL	++
V113	F	+++	++	++	10-15 mL	++

[a] Severity: +++ = marked; ++ = moderate; + = mild; neg = not observed.

Table 3 F03-09G-Phase-1 Natural History: Microscopic Findings

Animal ID	Sex	Lungs	Mediastinal lymph nodes, inflammation and/or edema	Bronchial lymph nodes, inflammation and/or edema	Mediastinal connective tissue[b]
V627	M	++[a]	++	++	++
V514	F	+++	++	++	neg
V569	M	+++	+++	+	neg
V113	F	+++	++	++	++

[a] Severity: +++ = marked; ++ = moderate; + = mild; neg = not observed

[b] Findings included one or more of the following: edema, inflammation, hemorrhage.

The objective of the non-GLP F03-09G study was achieved in that clinical signs were monitored, recorded and described such that they could be used in determining the clinical assessments of relevance to the animal model as well as establishing clinical parameter criteria to use for euthanasia criteria in subsequent antibacterial drug efficacy studies. Among the six animals on Phase 1 of the study, there was a strong correlation between fever, bacteremia and non-survival.

3.2.2 Natural History Study of Inhalational Plague Y. pestis Strain CO92 in Adult Telemetered African Green Monkeys (Study No. FY06-126)

The objective of this study (FY06-126) was to confirm the natural history and lethality of aerosolized *Y. pestis* in the AGM at an additional site, LRRI. This study was the first *Y. pestis* aerosol challenge in AGM performed at LRRI and therefore it was not intended to be compliant with GLP requirements. Good documentation and scientific practices were followed. Ten healthy, experimentally naïve, adult AGM (5 male, 5 female) weighing 3 to 6 kg were placed on study. Prior to challenge, the animals had telemetry devices implanted, as well as vascular access ports (Broviac catheters) for blood sampling. Baseline telemetry data were collected, with average readings every five minutes. Arterial blood gas, clinical chemistry, hematology, coagulation and *Y. pestis* microbiology were also assessed in the baseline period.

The challenge material was prepared by streaking slants of TBAB with yeast extract with *Y. pestis* CO92 and incubating at 28°C for 48 to 72 hours. Growth was collected with 1% peptone using sterile swabs, pooled and vortexed. Optical density measurements determined the concentration of organisms, which were then diluted to the appropriate concentration for the target challenge dose.

AGM Model of Pneumonic Plague

On the day of challenge, AGM were anesthetized and a baseline chest radiograph taken prior to challenge. Animals were then exposed to the *Y. pestis* aerosol in a head-only chamber in a Class III biosafety cabinet, with a target dose of 100 LD_{50} equivalents. Minute volume was measured by whole-body plethysmography in real time during the challenge. The duration of each aerosol exposure is directly related to the animal's minute volume to achieve the target challenge dose. Each animal's actual challenge dose was verified retrospectively by collecting the aerosol during the challenge in an all glass impinger and quantifying organisms by plating dilutions. Challenges were performed on two separate days three weeks apart, with five animals challenged each day.

Post-challenge, blood was drawn each day for quantitative bacteriology, hematology, clinical chemistry, arterial blood gas, and coagulation indices. Clinical signs were documented twice daily and telemetry recording continued every 5 minutes. Animals were infused with saline every twelve hours beginning one day after challenge, in order to establish procedures for subsequent efficacy studies; procedures for catheter care were refined in the second challenge cohort; these infusions are unique to this natural history study. When increased respiration was noted by telemetry, animals were anesthetized for another chest radiograph; eight of the animals had chest radiographs on Day 0, Day 3 and Day 4, while two animals only had chest radiographs on Day 0 and Day 3 (X790) or Day 0 and Day 4 (X789). Microbiology during the post-challenge period was measured quantitatively by plating of serial dilutions on tryptic soy agar (TSA) plates. Animals that were judged to be euthanized were first anesthetized for a chest radiograph and terminal blood was drawn for arterial blood gas, clinical chemistry, hematology, quantitative bacteriology and coagulation indices, prior to euthanasia. A complete gross necropsy was performed on all animals and all findings were recorded. Tissues taken at necropsy for terminal pathogen load were lung (lesion and non-lesion tissue), liver, spleen and tracheobronchial lymph nodes. Brain was collected for histopathology only.

Animals received between 44 and 255 LD_{50} equivalents with a mean dose of 135 ± 68 LD_{50} equivalents. All study animals became ill. Three animals died naturally and 7 were euthanized in a moribund condition (Table 4). The average challenge dose and standard deviation for the first challenge day was 190 $\pm$ 41 LD_{50} equivalents and for the second day was 79 $\pm$ 33 LD_{50} equivalents. All ten animals became bacteremic: three on Day 2 (all female), five on Day 3 (3 male, 2 female), and two at the time of death, Day 4 (both male). The animals that became bacteremic earliest demonstrated the highest levels of bacteremia and tended to be female (lower body weight). Females also tended to demonstrate higher numbers of bacteria in spleen and liver at necropsy. Fever was defined as a body temperature above 39°C for 2 hours, which correlated well with a 2°C increase over baseline body temperature, and was determined based on the data obtained in this study, and not defined prospectively.

The following tissues were collected at necropsy for terminal pathogen load: lung (lesion and non-lesion tissue), liver, spleen and tracheobronchial lymph nodes. The brain was also collected and fixed in 10% neutral buffered formalin for histopathology only. All animals on study had gross and microscopic lesions consistent with pneumonic plague (Table 5).

AGM Model of Pneumonic Plague

Bacterial contamination of blood samples taken via the Broviac catheter from live animals challenged on the first challenge day was problematic. Microbial characterization tests (coagulase and catalase) indicated that the contaminant was most likely a non-pathogenic *Staphylococcus* spp., presumably the normal flora microbe *S. epidermidis*. Blood samples drawn from the femoral artery and tissue samples at necropsy were not contaminated which suggested that the Staphylococcal contaminant may have come from a biofilm established on the Broviac catheter. A different Broviac catheter more suitable for sterile blood draws was used for animals challenged on the second challenge day and contamination was not an issue.

Table 4 FY06-126 Natural History: Challenge Dose, Survival, Fever and Bacteremia Observations

Animal ID	Sex	Challenge Dose, LD_{50} equivalents	1st Bacteremia, day	Time to Fever, hours[a]	Outcome[b]	Time to Death, hours[c]	Last Bacteremia, CFU/mL
X756†	M	255	4*	68	EU	95	1.7×10^2
X666†	F	206	3	75	EU	96	9.3×10^2
X538†	M	167	3	74	EU	93	2.1×10^2
X532†	M	163	3	87	EU	95	6.6×10^2
X705†	F	160	2	52	D	86	1.4×10^5◇
X789	F	129	3	74	D	99	$>2.0 \times 10^5$
X784	M	91	3	62	EU	100	1.3×10^3
X775	M	74	4*	79	EU	102	5.4×10^2
X774	F	58	2	52	EU	92	$>2.0 \times 10^6$
X790	F	44	2	52	D	71	$>2.0 \times 10^6$

† animals challenged on first challenge day
* first bacteremia was the terminal sample
◇ terminal sample not available; Day 3 result shown here
[a] Body temperature of 39°C for two consecutive hours was defined as the onset of fever
[b] EU = euthanized; D = died
[c] time to death as determined by telemetry data

Table 5 FY06-126 Natural History: Summary of Prominent Microscopic Findings

Animal ID	Lung inflammation	Pleural fibrosis	Tracheobronchial lymph node edema	Tracheobronchial lymph node bacteria	Spleen, bacteria	Liver, hydropic change	Brain
X756	+++[a]	-	+++	++++	-	++	U[b]
X666	+++	-	-	-	-	++++	U
X538	+++	-	-	+	-	+	U
X532	+++	+++	-	++	-	+++	U
X705	+++	-	+++	++++	+++		U
X789	++	++	+++	++	++++	+++	U
X784	+++	+++	-	++	-	++	U
X775	+++	-	-	+	-	+++	U
X774	+++	-	+++	++++	+++	-	U
X790	+++	+++	+++	+++	+++	-	U

[a] Severity: ++++ = marked; +++ = moderate; ++ = mild; + = minimal; - = finding not present or observed

[b] U = unremarkable organ/tissue

The study confirmed the progression of pneumonic plague in the AGM following aerosol exposure at LRRI. Fever and bacteremia were confirmed as consistent markers of disease for testing of antibacterial drug efficacy. Terminal bacteremia levels were somewhat lower than those seen in other studies, though the significance of this observation is unknown.

3.2.3 Natural Course of Untreated Pneumonic Plague in African Green Monkeys (Study No. 617-G607610)

The objective of this study (617-G607610) was the characterization of pneumonic plague disease through clinical observations, blood analyses and telemetric monitoring of physiological parameters of AGM infected with aerosolized *Y. pestis*. This study was also intended to confirm the course of disease seen in previous studies, and to establish this model in another laboratory. The assessments in this protocol were designed to determine the timing of therapy in future efficacy studies. Ten experimentally naïve, healthy, adult AGM (3 male, 7 female) weighing 3.3 to 4.7 kg were placed on study. Prior to challenge, the animals had telemetry devices implanted, and baseline telemetry data were collected, with average readings obtained every 15 minutes for at least seven days. Clinical observations were recorded daily. Clinical chemistry and hematology were also assessed prior to challenge, on Day 0. A memory B-cell assay was also performed to ensure that animals were naïve for exposure to *Yersinia* antigens; some very low values were observed that were considered background and therefore no animals were excluded from study.

AGM Model of Pneumonic Plague

The challenge material was prepared by first streaking *Y. pestis* CO92 on Congo red agar and heart infusion agar for isolation. After incubation at 26 $\pm$ 2°C for 66 hours, an isolated colony positive for the pigmentation locus in *Y. pestis* (Pgm+) was selected from the Congo red agar plate and used to inoculate HIB without $CaCl_2$ and incubated in a shaker incubator until reaching a target concentration, measured by optical density at 600 nm. The bacteria were centrifuged, washed and resuspended in BSGT (buffered saline with 0.01% gelatin and 9.7% trehalose) at the target concentration for aerosolization.

On the day of challenge, AGM were anesthetized and then exposed to the *Y. pestis* aerosol in a head-only chamber in a Class III biosafety cabinet, with a target dose of 100 LD_{50} equivalents. Minute volume was measured by whole-body plethysmography in real time during the challenge. Each animal's actual challenge dose was verified retrospectively by collecting the aerosol during the challenge in an all glass impinger and quantifying organisms by plating serial dilutions. All animal challenges were performed on a single day.

After challenge, clinical observations were recorded twice daily along with continuous telemetry monitoring. Blood was obtained daily for bacteremia, hematology and clinical chemistry. Bacteremia was determined by serial dilution and plating on TSA for quantitation. Samples were also streaked directly on Congo red agar, for qualitative assessment of colony morphology and pigmentation consistent with *Y. pestis*. Terminal blood samples were assessed qualitatively for *Y. pestis* bacteria.

Actual challenge doses ranged from 106 to 1150 LD_{50} equivalents with a mean of 613 $\pm$ 386 LD_{50} equivalents (Table 6). The spray factor, or the ratio between the aerosol concentration and the nebulizer concentration, varied by a log on this study. The starting nebulizer concentration also varied by more than a log, and accounts for a significant amount of the animal to animal variability in challenge dose. Subsequent studies investigated the aerosolization efficiency to bring it under tighter control and are not the subject of this summary. This study is being included here in support of the general disease pathogenesis.

Table 6 617-G607610 Natural History: Challenge Dose, Survival, Fever and Bacteremia Observations

Animal ID	Sex	Challenge Dose, LD_{50} equivalents	1st Bacteremia, day	Time to Fever, hours[a]	Outcome[b]	Time to Death, hours[c]	Last Bacteremia, CFU/mL[d]	Terminal Blood Culture Result[b]
X396	M	1150	2	41.2	D	61.6	$6.57x10^5$	pgm^+, C
X106	M	1094	2	38.8	D	85.8	$7.70x10^6$	pgm^+
X515	F	1024	2	36.6	D	68.9	$5.97x10^4$	pgm^+, C
X434	F	836	2	48.2	D	64.2	$8.47x10^3$	pgm^+, C
X421	F	568	2	45.4	D	67.4	$3.76x10^3$	pgm^+, C
X711	F	516	2	44.9	D	68.8	$4.37x10^4$	o, C
X770	F	322	2	43.0	D	66.0	$6.23x10^4$	pgm^+, C
X759	F	288	2	45.7	D	90.4	$2.14x10^7$	pgm^+
X511	F	233	2	49.5	D	65.5	$3.97x10^4$	pgm^+, C
X606	M	106	2	44.5	D	89.2	$2.54x10^4$	pgm^+

[a] Fever was defined as the first of three consecutive hourly measurements at least 1.5°C above their respective baseline averages at the same hour of the day. For Animal X515 some telemetry readings were not available. The time reported was the first time a three hour block was over the limit, but the middle hour measurement was missing

[b] C = contamination present; D = died; o = negative for pigmented *Y. pestis;* pgm^+ = positive for pigmented *Y. pestis*

[c] time to death was taken from telemetry data or clinical observation

[d] last bacteremia is from the last daily blood draw, not a terminal sample

Animals on this study were all bacteremic by the Day 2 blood draw, ranging from 33 to 657,000 CFU/mL (Table 6). A terminal sample was taken from all animals and was analyzed only by qualitative streaking on Congo red agar; nine of the terminal samples contained pigmented *Y. pestis* (all except X711). Terminal nasopharyngeal discharge and lung fluid were also analyzed by streaking on Congo red agar and all samples obtained were positive for *Y. pestis*. Contamination with unidentified bacteria was observed in 7 of the terminal blood samples, 3 of the nasopharyngeal washes, and one of the lung washes. The source and nature of the bacterial contaminant(s) were not identified in this study.

Fever was not prospectively defined for this first pneumonic plague study in this laboratory. Telemetry recordings indicated that all animals initiated a fever between 1.5 and 2.0 days, with a sustained body temperature of about 41°C around 2 days post-challenge. Heart rate increased around 2 days post-challenge, accompanied by slight increases in blood pressure and a marked increase in respiratory rate. All animals died, between 2.5 and 4.0 days post-challenge. Based

on the clinical symptomatology, the cause of death in all ten animals was concluded to be pneumonic plague.

A complete gross necropsy was performed on all animals and all findings were recorded. The most prominent pathologic findings are recorded in Table 7. The following tissues were collected at necropsy and fixed in 10% neutral buffered formalin: lungs, intrathoracic lymph nodes, and gross lesions. Collected tissues were examined histopathologically as needed to confirm the cause of death. There were no findings in brain following microscopic examination in any animals. Animal X106 was noted to have fibrin thrombi in renal glomerular capillaries, consistent with disseminated intravascular coagulopathy.

Table 7 617-G607610 Natural History: Summary of Prominent Gross and Microscopic Findings

Animal ID	Sex	Challenge Dose, LD_{50} equivalents	Gross Findings		Microscopic Findings					
			Lung, discoloration/nodule/mass	Thoracic Cavity fluid, mL	Lung, inflammation	Lung, bacteria[b]	Bronchial Lymph Node, bacteria	Mediastinal Lymph Node, bacteria	Spleen, bacteria	Kidney, glomerulus, fibrin
X396	M	1150	NGO	15	++	++/+++	+++	++++	+++	NMO
X106	M	1094	++[a]	15	+++	++/++++	++++	++++	NMO	+
X515	F	1024	NGO	3	++	++/++++	+++	++	NMO	NMO
X434	F	836	NGO	20	++	++/+++	+++	++	+++	NMO
X421	F	568	NGO	7	++	++/++	++++	++	++++	NMO
X711	F	516	NGO	15	++	++/+++	++	++	+	NMO
X770	F	322	NGO	10	++	++/+++	++++	+++	++	NMO
X759	F	288	++	10	++++	+/++++	++	NMO	+	NMO
X511	F	233	NGO	15	++	+/++	+++	++	+++	NMO
X606	M	106	++++	NGO	++++	++/++++	++	++	++	NMO

[a] Severity: ++++ = marked; +++ = moderate; ++ = mild; + = minimal; NGO = no gross observation; NMO = no microscopic observation

[b] † observations are intracellular/extracellular bacteria.

The progression of untreated pneumonic plague in AGM following aerosol exposure to *Y. pestis* was confirmed in the first, non-GLP study at BBRC. The challenge dose exceeded the target in all but one animal; however the clinical signs and pathology are the same as that reported in the

other natural history studies, though the time course of disease is somewhat shorter. Animals presented with fever earlier than in other studies and the average time to death was somewhat shorter, Day 3 rather than Day 4. This study was repeated after additional work to refine the aerosol challenge conditions to achieve the target challenge dose.

3.2.4 Natural Course of Untreated Pneumonic Plague in African Green Monkeys (Study No. 875-G607610)

The objective of this study (875-G607610) was the assessment of pneumonic plague disease through clinical observations, blood analyses and telemetric monitoring of physiological parameters of AGM infected with aerosolized *Y. pestis*. This study was intended to confirm the course of disease seen in previous studies with a target challenge dose of 100 LD_{50} equivalents, in preparation for antibacterial drug efficacy studies. Ten experimentally naïve, healthy, adult AGM (5 male, 5 female) with target weights of 3.5 to 6.0 kg were placed on study. Prior to challenge, the animals had telemetry devices implanted, and baseline telemetry data were collected, with average readings obtained every 15 minutes for 13 days. Clinical observations were recorded twice daily. Clinical chemistry, hematology and coagulation indices were also assessed prior to challenge, on Day 0. A *Yersinia* antigen enzyme-linked immunosorbent assay (ELISA) was also performed to ensure that animals were naïve to *Yersinia*; no animals were excluded from study.

The challenge material, *Y. pestis* CO92, was prepared in the same manner as Study 617-G607610. Briefly, pigmented colonies from fresh Congo red agar plates were inoculated into heart infusion broth and grown to saturation at 26°C. Bacteria were washed and resuspended at the desired concentration and held on ice prior to aerosolization.

On the day of challenge, an aerosol pre-run was performed without an animal, and the resulting aerosol was analyzed by flow cytometry after staining with a fluorescent vital dye. AGM were anesthetized and then exposed to the *Y. pestis* aerosol in a head-only chamber in a Class III biosafety cabinet, with a target dose of 100 LD_{50} equivalents. Minute volume was measured by whole-body plethysmography in real time during the challenge. Each animal's actual challenge dose was verified retrospectively by collecting the aerosol during the challenge in an all glass impinger and quantifying organisms by plating dilutions. All animal challenges were performed on a single day over a three hour period.

After challenge, clinical observations were recorded three times daily along with continuous telemetry monitoring. Blood was obtained daily for bacteremia, hematology, clinical chemistry and coagulation. Bacteremia was determined both quantitatively, by serial dilution and plating on tryptic soy agar, and qualitatively, by direct streaking on Congo red agar and *Yersinia* selective agar. Blood was also mixed with tryptic soy broth and incubated for streaking of enriched cultures on Congo red and *Yersinia* selective agar.

AGM Model of Pneumonic Plague

Actual challenge doses delivered in this study ranged from 24 to 88 LD_{50} equivalents with a mean of 48 ± 23 LD_{50} equivalents. These data are summarized in Table 8 along with fever, bacteremia and survival data. The individual animal spray factors, and therefore challenge doses, were much tighter on this study than Study 617-G607610. Based on clinical symptomatology, all ten animals were concluded to have succumbed to pneumonic plague. On Day 2, three animals were bacteremic by both quantitative and qualitative assessment, whereas an additional 4 animals were bacteremic by qualitative assessment only. Fever, as defined by an increase of >1.5°C over baseline, was seen between 48 and 62 hours in all animals. All animals died naturally between Day 3 and Day 6 and underwent gross necropsy and histopathology of selected tissues.

Table 8 875-G607610 Natural History: Challenge Dose, Survival, Fever and Bacteremia Observations

Animal ID	Sex	Challenge Dose, LD_{50} equivalents	1st Bacteremia, day[a]	Time to Fever, hours[b]	Outcome[c]	Time to Death, hours	Terminal Bacteremia, CFU/mL
Y256	M	88	2	50	D	66.3	$1.03x10^9$
X753	F	76	3	61	D	102.6	$7.43x10^6$
Y213	F	76	3	58	D	77.6	$2.41x10^8$
X486	M	43	2†	59	D	87.5	$8.03x10^7$
X603	F	38	2†	52	D	88.2	$2.16x10^9$
W904	F	38	2~	55	D	112.3	$3.43x10^5$
X900	M	37	3	62	D	139.0	$8.63x10^6$
Y212	F	36	2~	56	D	74.0	$4.73x10^7$
X950	M	25	2	51	D	84.7	$1.16x10^9$
X840	M	24	2	48	D	100.4	$7.23x10^7$

[a] by both qualitative and quantitative methods, unless indicated otherwise, † positive by both methods but below 250 CFU/mL, ~ positive only by qualitative method

[b] Fever was defined as body temperature >1.5°C above baseline average

[c] D = died naturally

A complete gross necropsy was performed on all animals and all findings were recorded. The following tissues were collected at necropsy and fixed in 10% neutral buffered formalin: lungs and bronchi, bronchial and mediastinal lymph nodes and gross lesions. These tissues were evaluated microscopically. Selected gross and microscopic findings are summarized in Table 9.

AGM Model of Pneumonic Plague

Prominent findings seen in 9 of 10 animals at gross necropsy were thoracic cavity fluid and observations such as foci, discoloration, nodules and/or masses in lungs. Microscopic findings included bacteria in all animals in lungs as well as tracheobronchial and mediastinal lymph nodes. Only one animal exhibited macroscopic findings in the brain, confirmed microscopically by the presence of bacteria.

Table 9 875-G607610 Natural History: Summary of Prominent Pathologic Findings

Animal ID	Sex[a]	Challenge Dose, LD_{50} equivalents	Gross Findings		Microscopic Findings			
			Thoracic Cavity fluid (mL)	Lung: focus, discoloration, nodule, mass[b]	Lung, bacteria	Bronchial Lymph node, bacteria	Mediastinal lymph node, bacteria	Brain, meninges, bacteria with fibrin exudation
Y256	M	88	10	Y	++[d]	+++	+	NE[c]
X753	F	76	30	Y	+++	+++	++	NE
Y213	F	76	40	N	+	++	+++	NE
X486	M	43	40	Y	++	+	+++	NE
X603	F	38	8	Y	++	+++	+	NE
W904	F	38	NGO[e]	Y	++++	++	++	NE
X900	M	37	10	Y	++++	++++	++	NE
Y212	F	36	30	Y	+	++	++	NE
X950	M	25	30	Y	++	+++	++	+/++
X840	M	24	25	Y	++++	++	+	NE

[a] F = female; M = male.
[b] Y = yes, N = no.
[c] NE, not examined.
[d] Severity: ++++ = marked; +++ = moderate; ++ = mild; + = minimal.
[e] NGO = no gross observation.

The progression of untreated pneumonic plague in AGM following aerosol exposure was documented in this non-GLP study. Animals presented with fever and bacteremia in a time

frame similar to other natural history studies conducted with this challenge dose. This target challenge dose results in a disease time course that could potentially be effectively treated with timely administration of effective antibacterial drugs.

3.3 Summary of the African Green Monkey (AGM) Model of Pneumonic Plague

In summary, the series of studies described here are the first studies conducted to establish the non-human primate (AGM) animal model for the evaluation of antibacterial drug treatment of pneumonic plague disease. The objectives of the studies conducted in support of the animal model were successfully achieved and established the natural history of disease progression in the AGM. Independent radiology review of the radiographs from two natural history studies (Appendix B) confirms that there is a common radiographic appearance and progression of severity of changes over time associated with lethal infection by inhaled *Y. pestis* Strain CO92 in African Green Monkeys. Independent pathology review (Appendix A) of the microscopic slides from the four natural history studies confirms that there is a common pathology associated with lethal infection by inhaled *Y. pestis* Strain CO92 in African Green Monkeys. The prominent characteristics and pathology of pneumonic plague are consistent in the African Green Monkey across laboratories.

4 Comparison of Human and Animal (African Green Monkey) Natural Courses of Pneumonic Plague – DMID Perspective

DMID/NIAID has studied the exposure of AGM to *Y. pestis* in several nonclinical studies as described above. In order to support that the AGM is a well-characterized animal model for predicting response in human pneumonic plague, a comparison of the characteristics of human disease using the literature data presented above and the data obtained in the nonclinical studies with regard to clinical signs and symptoms, pathophysiology, disease progression, radiology, and histopathology are presented in this section. A fair comparison of natural cases of pneumonic plague in humans to experimental infections of AGM requires recognition of the limitations of comparisons and differences between these diseases. The human cases summarized are based on the diagnosis of pneumonic plague rather than route of exposure. While the route of exposure and timing can be surmised from some of the human case reports, it is not always known. For human cases of inhaled *Y. pestis*, the dose is unknown, while the dose in AGM was measured. The inhaled dose of *Y. pestis* varies from animal to animal based on respiratory minute volume; however, it is likely to be equal to or greater than the level of exposure in the human cases summarized above. The timing of signs and symptoms in human cases are sometimes described relative to the first sign or symptom and sometimes relative to the time of presumptive exposure; whereas, timing in the animal studies is always relative to exposure. Clinical symptomatology, such as headache, chills, and myalgia, are not obtainable in animal studies, and therefore, no comparison can be made. While descriptions of the course of human disease demonstrated

variability in presentation or care-seeking behavior, the animal studies are conducted in controlled, laboratory settings which results in less apparent variability as presentation is not a variable.

In Wu Lien-Teh's 1926 treatise (Wu Lien-Teh, 1926), the time course of human disease was reported to be 2 to 9 days, with the majority of cases being 2 to 5 or 6 days. All other case studies presented here fit within the overall time frame of 2 to 9 days. This time course matches exactly that seen in the AGM studies summarized here and in Davis et al, 1996 (i.e., 2 to 9 days).

Fever is a clinical sign noted consistently in both humans and AGM. Among the human cases summarized in this document, only a few descriptions made no mention of fever: Dr. Manser's nurse, and two of the three refugees whose descriptions only refer to suspicion of plague or the lack of plague-like symptoms. All other cases noted a fever. This is consistent with the AGM model, in which all animals present with a fever, typically at 72 hours post-exposure. The most compelling human case description that determined the interval between exposure and fever is the wildlife biologist exposed during necropsy of a mountain lion, where the time to fever was 3 days (Wong, 2009). This coincides with the time to fever seen in the AGM studies.

Most human cases presented here were confirmed as pneumonic plague based on the presence of *Y. pestis* bacilli found in sputum rather than blood. The AGM model correlated fever with the presence of bacteria in the blood; however, observations in these studies of sputum or frothy discharge from nares, with or without blood, are consistent with the progression of cough in human disease. Both of the Battelle natural history studies cultured fluid from the lung and nasal discharge for the presence of *Y. pestis.* In all 20 animals, *Y. pestis* was found at death in both nasal discharge and lung fluids.

Heart rate and respiratory rate were noted in the clinical summaries to be elevated during the course of disease, particularly in the cases followed by Chun and Wu Lien-Teh (Chun [a, b], 1936; Wu Lien-Teh, 1926). These observations were typically made daily, while in the AGM studies, they were monitored continuously via telemetry, except for respiratory rate in study F03-09G, which was measured visually, though frequently. The increased respiratory rate tends to be more dramatic in late disease in both human and AGM cases. Heart rate is also increased in both humans and AGM.

In humans, changes in pulmonary function were mainly gathered through auscultation. Although auscultation was not performed in the AGM, the character of respiration was assessed during scheduled and frequent observations. Rales were observed in both humans and AGM; 3 of 4 diseased animals in study F03-09G had rales just prior to euthanasia/death. Chest radiographs were performed in 2 AGM studies (F03-09G and FY06-126) and were summarized in NIAID's Independent Review of Radiology (NIAID-Yp-NatHis-Rad-2011). Pulmonary infiltrates in AGM were mild to moderate on Day 3 and severe at the time of death. This is consistent with the clinical radiological findings reported by Alsofrom et al. (Alsofrom 1985), where 8 of 9 cases were reported as bilateral pulmonary infiltrates, compared to approximately 65% in AGM.

AGM Model of Pneumonic Plague

There are few published reports of human pathology (Doll, 1994; Werner, 1984; Wong, 2009), and macroscopic and microscopic pathology findings are very similar in human and AGM cases of pneumonic plague. Pathology findings reported in humans were: lobar to sublobar pulmonary consolidation (3 of 3), inflammatory infiltrates (neutrophils with fibrin) (2 of 3), hemorrhagic and frothy fluid in both lungs (1 of 3), pulmonary exudates and effusions (1 of 3), bronchopneumonia (1 of 3), and the presence of bacilli (2 of 3). Note that these findings are taken directly from a small number of published reports and were not independently determined. The most prominent pulmonary findings in the independent review of 36 untreated AGM were bacteria, edema, hemorrhage, inflammatory infiltrate (intra-alveolar neutrophils followed by macrophages), and pleural fibrin. These pathology findings are essentially indistinguishable.

Table 10 summarizes a comparison of the main features of pneumonic plague between humans and AGM.

Table 10 Human and African Green Monkey Natural Courses of Pneumonic Plague

	Human[a]	**African Green Monkey[b]**
Time course of disease, days	2 to 9	2 to 9
Temperature	Elevated in ~100% of cases (at 3 days in 1 case)	Elevated in 100% of cases (typically 3 days post-exposure)
Yersinia pestis present	Positive in 100% of sputum	Positive in 100% of blood and/or lung/nasal fluids
Heart rate	Elevated	Elevated[c]
Respiration rate	Elevated late in disease	Elevated late in disease
Chest radiographs	Pulmonary infiltrates 90% bilateral	Pulmonary infiltrates Approximately 65% bilateral[c]
Pathology, lung	Consolidations, Inflammatory infiltrates, Hemorrhagic/frothy fluid, Exudates and effusions, Bronchopneumonia, Bacilli	Bacteria, Edema, Hemorrhage, Inflammatory infiltrates/bronchopneumonia, Pleural fibrin

[a] Data from 3 cases in 3 publications (Doll, 1994; Werner, 1984; Wong, 2009)
[b] Data from 34 untreated AGM from 4 studies (F03-09G, FY06-126, 617-G607610, and 875-G607610) and Davis, 1996
[c] Heart rate and radiograph data from F03-09G and FY06-126

Clinical signs/tests that could potentially serve as a trigger for treatment were bacteremia, body temperature, heart rate, and respiratory rate; chest radiographs were also considered though are not possible at all study sites. Body temperature, heart rate, respiratory rate and chest radiographs can be real-time triggers, but bacteremia requires at least 24 hours of culture. It is also worth noting that bacteremia and chest radiographs are limited in frequency due to animal welfare concerns about blood volume and anesthesia, while body temperature, heart rate and respiratory rate, were monitored continuously by telemetry. In practice, chest radiographs were obtained upon the appearance of other signs such as increased respirations or body temperature. Body temperature, heart rate, respiratory rate and chest radiographs are all general signs and only bacteremia is specific to *Yersinia pestis* infection. Therefore, it was important to determine the correlation of bacteremia with other clinical signs. Comparing body temperature, heart rate and

respiratory rate as possible treatment triggers, body temperature increased significantly and maximally early, while increases in heart and respiratory rates began at the same time and continued to increase as disease severity progressed, with maximal levels appearing late in disease. Two challenged survivors never exhibited increased body temperature, heart or respiratory rates and were also never bacteremic. Initial chest radiographs, at the time of first clinical signs (fever) were abnormal (predominately mild) and increased in severity until death (moderate to marked/severe). Therefore, fever was selected as the treatment trigger for subsequent studies of antibacterial drug efficacy.

5 Pneumonic Plague – Natural History in African Green Monkey (AGM) Model and Comparison to Human Disease – DAIP Perspective

In order to be able to develop a product for the treatment of pneumonic plague, it is important to identify and characterize animal models of pneumonic plague to be used to evaluate a product's efficacy. Specifically, as provided in the Animal Rule, it was important to study the natural history of plague infection in the animal model, with the goal of understanding the pathophysiological mechanism of the toxicity of the substance (Y. pestis) and prevention or substantial reduction of disease and/or mortality by the product.

The AGM is proposed to be used as an animal model for evaluating efficacy of products for treatment of pneumonic plague under the Animal Rule, 21 CFR 314.610, Subpart I.

The AGM model of primary pneumonic plague has been evaluated in 4 natural history studies conducted by 3 independent laboratories sponsored by NIAID.

5.1 Natural History Studies of Pneumonic Plague in African Green Monkeys

The design was fairly similar between the studies. Experimentally naïve adult symptom, malformation, and specific pathogen free AGM of both sexes were obtained from St. Kitts West Indies. In Study 617-G607610 and Study 875-G607610 (Battelle), in addition to viral screens animals were evaluated for previous exposure to *Y. pestis* and environmental *Yersiniae.* Animals were initially quarantined, then surgically implanted telemetry devices and allowed to recover for 14 days post surgery. *Y. pestis* challenges were conducted in Biosafety Level 3 (BSL-3) containment laboratories. *Y. pestis* strain CO92 was the challenge agent in all studies. The organism was administered via a nebulized aerosol in the head-only exposure chamber with a flow rate of ~16L/min. All animals were anesthetized and challenged with a targeted at 100 times the LD_{50}. However, the actual challenge dose received varied widely within and between the studies. Animals in Study F03-09G (USAMRIID) received the lowest mean challenge dose. As a result 2/6 animals in Study F03-09G did not develop the disease and survived the experiment.

After challenge, animals were observed for signs and symptoms of the disease twice daily. Data analyses of telemetry recordings were limited to temperature and heart rate in Study F03-09G

(USAMRIID), to temperature, heart rate and respiratory rate to temperature in Study FY06-126 (LRRI) while in Study 617-G607610 and Study 875-G607610 (Battelle), temperature, heart rate, respiratory rate and blood pressure were analyzed. In Study F03-09G (USAMRIID) respiratory rates were recorded manually every 4 hours beginning at 42 hours postexposure. Weight, blood culture, CBC, chemistry data were collected daily. Coagulation parameters, arterial blood gas, chest X-ray data were collected in some studies.

While in Study 617-G607610 and Study 875-G607610 (Battelle) and Study FY06-126 (LRRI) clinical observations were descriptive, in Study F03-09G (USAMRIID) a euthanasia scoring chart was used to monitor clinical parameters and to determine a need for euthanasia. After natural death or euthanasia all animals underwent necropsy and microscopic examination.

Table 11 summarizes findings from the natural history studies of primary pneumonic plague in AGM.

Table 11 Summary Findings, AGM Primary Pneumonic Plague Natural History Studies

Parameter	**Battelle Study 617-G607610**	**Battelle Study 875-G607610**	**LLRI FY06-126**	**USAMRIID F03-09G**
Average *Y. pestis* Exposure Dose of (range)	614 LD_{50} (106 LD_{50} to 1150 LD_{50})	48 LD_{50} (24 LD_{50} to 88 LD_{50})	134 LD_{50} (44 LD_{50} to 255 LD_{50})	9 LD_{50} to 57 LD_{50}
Animals	3 males and 7 females	5 males and 5 females	5 males and 5 females	3 males and 3 females
Mortality	100% 10 found dead	100% 10 found dead	100% (3 found dead, 7 euthanized)	67% (4/6: 1 found dead, 3 euthanized)
Average Time to Death (range)	72 hours (61 to 90 hours)	93.5 hours (67.5 to 139 hours)	90 hours (72 to 100 hours)	112 hours (99.5 to 125 hours)
Average Time to Fever Onset (range)	44 hours (39 to 49.5 hours)	54 hours (47 to 61.5 hours)	64 hours (46 to 80.5 hours)	73 hours (70 to 76 hours)
Average Interval between Fever and Death (range)	28.5 hours (16 to 44.5 hours)	39 hours (17.5 to 78 hours)	26 hours (16 to 42 hours)	38.5 hours (29.5 to 51 hours)
Initial Level of Bacteremia (cfu)	33 to >650,000 cfu/mL	600 cfu to >7,000,000 cfu/mL	10 cfu to >200,000 cfu/mL	3×10^6 to 9×10^8 cfu/mL (terminal)
Timing of Bacteremia Onset (range)	48 to 72 hours (100% + by Day 2)	43.5 hours (30 to 68.5 hours)	66.5 hours (43 to 94 hours)	60 hours (48 to 72 hours)
Radiographic abnormalities	n/a	n/a	5/10 on Day 3	4/4 at 80 - 83h postexposure
Symptoms	Fever, loss of appetite, respiratory distress, bloody respiratory secretions, lethargy	Fever, hunched posture, respiratory distress, bloody respiratory secretions, lethargy	Fever, loss of appetite, diarrhea, lethargy	Fever, loss of appetite, respiratory distress, lethargy
Pathology	Hemorrhagic pneumonia	hemorrhagic pneumonia	fibrinosuppurative hemorrhagic pneumonia	fibrinosuppurative hemorrhagic pneumonia

5.2 Comparison of AGM model of pneumonic Plague to Human Disease

Review of the natural history studies of pneumonic plague in AGM and comparison of critical elements of the AGM model to human primary pneumonic plague cases available for review in the public domain revealed similarities between the animal and human disease. The AGM model performed consistently in 3 different laboratories with some variations as discussed below.

The **Challenge agent** used in all 4 studies was *Y. pestis*, biovar *orientalis*, strain CO92, recovered from a person with pneumonic plague.[3] This biovar has been commonly isolated from humans with plague during the last century and remains a relevant human pathogen.[4]

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

Close contact with an infected human or animal in later phase of the disease, characterized by the production of highly infectious hemorrhagic sputum has been simulated in AGM model by head only *Y. pestis* aerosol exposure.

The **Target aerosol** of 100 x LD_{50} for AGM is many times greater than the estimated infectious inoculum in humans of 100-500 cfu[6]. The data from 4 AGM natural history studies reveals that despite significant variability in the actual challenge dose received (9 to >1000xLD_{50}), exposures > 20 x LD_{50} induce signs and symptoms of pneumonic plague and are uniformly fatal. In outbreak investigations the persons in close contact with the index case in the late phase of their disease were the most susceptible. Airborne droplet transmission is hypothesized for pneumonic form of the disease in humans. *Y. pestis* could be bioweaponized with an infectious aerosol delivered over a large, densely populated geographic area resulting in high exposure/disease rates.[7]

The **Incubation period** for primary pneumonic plague on average is 3 days and is similar between humans and AGM as is the duration of illness prior to death, averaging about 3 days. Calculation and comparison of the time course of the disease postexposure is feasible due to the characteristic abrupt onset of **signs/symptoms** – chills and fever in humans and fever in AGM. It is noteworthy that the temperature in AGM rises sharply and invariably persists until the time of

---

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

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

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

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

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

death. Malaise and chest pain reported in humans cannot be readily assessed in animals due to inability to verbalize; however, cough, tachycardia and tachypnea, direct signs of cardiovascular and respiratory organ system involvement, are manifested by both species as disease progresses. Increase in respiratory rate first was noted in animals after approximately 48 hours postexposure and coincided with onset of fever. Animals started to exhibit less variability in signs of respiratory distress (tachypnea and increased work of breathing) 24-36 hours prior to death. Extensive parenchymal inflammation of the lung tissue and cardiovascular collapse with coagulation abnormalities lead to death in men and AGM with primary pneumonic plague. Neutrophilic leukocytosis, metabolic acidosis, elevation of liver enzymes and creatinine as well as prolonged PT/PTT were observed in some animals late in the disease and correspond to metabolic, hematologic and coagulation abnormalities seen in humans with primary pneumonic plague.

Due to the sporadic nature of the disease in man, particularly in the US, **diagnosis of plague** in an index case requires a high index of suspicion with careful attention to the exposure history and in many cases is established postmortem due to the fulminant nature of the disease (median time to death 3 days) and the limitations of bacteriological identification (3-4 days culture turnaround time). In cases with survival outcome, the diagnosis was either suspected early during outbreak investigation and/or patients were treated empirically shortly after disease onset with broad spectrum antibacterial drugs for pneumonia allowing patients to survive to modification of therapy after pathogen isolation and identification. In the AGM model, the **therapeutic window for intervention**, i.e. time from onset of disease as manifested by fever (definition of fever varied between 4 natural history studies from 39°C to >1.5 °C elevation over a baseline or over corresponding hourly baseline for 2-3 consecutive hours) is on average 2 days, while in humans this average appears to be a day longer. Differences between the studies in the definition of fever **as the trigger for intervention** could be considered minor as the timing of the onset of fever as defined by individual studies generally differed from one another by 3 hours or less if applied to the same temperature recording. In addition, in some animals (30-50% depending on the study) death occurred in less than 24 hours after onset of fever unrelated to the challenge dose. All animals that developed fever after challenge were bacteremic by day 2-4 of illness and also at the time of death. As microbiology evaluations in AGM natural history studies were done infrequently (once daily), correlation between onset of fever and onset of bacteremia cannot be assessed precisely. However, up to 50% of animals were found to be bacteremic prior to onset of fever and quantitative blood cultures exhibit exponential increase in bacterial counts over time, suggesting that animals that were not bacteremic prior to onset of fever are likely to be positive at the time of fever onset. Real time confirmation of bacteremia prior to treatment is not feasible using current technologies due to delay in culture results.

Table 12 represents the summary of nonclinical and clinical data from the published literature as well as the collected nonclinical data from natural history and animal efficacy studies, and compares the similarities and differences of plague infection, host susceptibility and response,

pathophysiology, triggers for intervention, and measures of outcome between human disease and the AGM model of disease.

Table 12 Interspecies Comparison

DATA ELEMENTS	African Green Monkey (AGM)	Human
A. Characteristics of the CBRN Agent that Influence the Disease or Condition		
1. The challenge agent	*Y. pestis* CO92 strain at proposed inhalational dose of 100±50 x LD_{50}	*Y. pestis* (CO92 strain was isolated from a human with pneumonic plague). Infectious inoculum 100-500 organisms variable level direct inhalational exposures anticipated in contacts of pneumonic plague patients depending on the proximity and duration of contact. During a bioterrorism event, exposures might achieve several times the lethal dose.
2. Pathogenic determinants	F1 capsular antigen, V antigens and Yersinia outer membrane proteins (yops) antigens, plasminogen activator (Pla)	Same
3. Route of exposure	Aerosol (head only exposure)	Aerosol (direct aerosol exposure to a close contact with pneumonic plague or bioweaponized aerosol)
4. Quantification of exposure	100±50 x LD_{50} in AGM is much greater than the infectious inoculum in humans	Infectious inoculum 100-500 organisms. Variable level during close aerosol contact, high level possible if bioweaponized
B. Host Susceptibility and Response to Etiologic Agent	highly susceptible	highly susceptible
C. Natural History of Disease: Pathophysiologic Comparability		
1. Time to onset of disease/condition	26-80 hours (1-3) days to fever onset	1-6 days to fever and other symptom onset
2. Time course of progression of disease/condition	Time to death 15-75 hours (1-3 days) (median 2 days)	With no antibacterial drugs survival 2-6 days (median 3 days)
3. Manifestations (signs and symptoms)	Fever, hunched posture, inappetence, lethargy, tachypnea, tachycardia, cough, bloody respiratory secretions, neutrophilic leukocytosis, coagulation abnormalities	Abrupt onset of fever, chills, lethargy, headache, myalgia, arthralgia, vomiting, diarrhea, cough, hemoptysis, followed by respiratory distress, ARDS, DIC, multiorgan failure, shock, neutrophilic leukocytosis, thrombocytopenia, acidosis CXR: multilobar consolidation, cavities or bronchopneumonia
4. Pathology	Hemorrhagic pneumonia	Hemorrhagic pneumonia

AGM Model of Pneumonic Plague

DATA ELEMENTS	African Green Monkey (AGM)	Human
D. Trigger for Intervention	Fever (>1.5 C over baseline sustained for 2 hours)	Variable depends on high index of suspicion, positive history, evidence of pneumonia, hemorrhagic pneumonia; positive smear or culture, titers of anti-F1 antibodies
E. Characterization of the Medical Intervention		
1. Product class	Antibacterials	
2. Mechanism of action	Product class dependent	
3. In vitro activity	Product dependent	
4. Activity in disease/condition of similar pathophysiology	TBD	TBD
5. PK in unaffected animals/humans	TBD	TBD
6. PK/PD in affected animals/humans	TBD	TBD
7. PK interactions with medical products likely to be used concomitantly	TBD	TBD
8. Synergy or antagonism of medical products likely to be used in combination	TBD	TBD
F. Design Considerations for Animal Efficacy Studies		
1. Endpoints	Mortality	Mortality
2. Timing of intervention	after fever onset	Variable upon suspicion, time of diagnosis, or general condition -- empiric
3. Route of administration	IV, PO	IV, PO
4. Dosing regimen	Humanized	Intended to treat serious infections

6 Points for Discussion

Based on the information regarding the natural history of pneumonic plague (disease due to *Yersinia pestis*) in African Green Monkeys, and the information about human cases of pneumonic plague:

1. Please comment on the similarities and differences between the African Green Monkey animal model of pneumonic plague and human disease with regard to:
    a. Signs and symptoms
    b. Outcome
    c. Histopathology

    Please discuss if the AGM model is likely to predict the response in humans with pneumonic plague.

2. Discuss the manifestation(s) of pneumonic plague in the African Green Monkey animal model that could be used to determine the need for therapeutic intervention and its (their) relevance in treatment of human disease.

7 References

Alsofrom DJ, Mettler FA, Mann JM. Radiologic manifestations of plague in New Mexico, 1975-1980: A review of 42 proved cases. Radiology 1985;139:561-5.

Begier EM, Asiki G, Anywaine Z, Yockey B, Schriefer ME, Aleti P, et al. Pneumonic plague cluster, Uganda, 2004. Emerg Infect Dis 2006;12(3):460-7.

Bogen E. The pneumonic plague in Los Angeles. Cal West Med 1925;23:175-6.

Burmeister RW, Tigeritt WD, Overholt EL. Laboratory-acquired pneumonic plague: report of a case and review of previous cases. Ann Int Med 1962;56(5):789-800.

Chun JWH [a]. Clinical features. In Wu Lien-Teh, Chun JWH, Pollitzer R, and Wu CY. Plague: A manual for medical and public health workers. Shanghai Station, China: Weishengshu National Quarantine Service; 1936:309-333.

Chun JWH [b]. Therapy and personal prophylaxis. In Wu Lien-Teh, Chun JWH, Pollitzer R, and Wu CY. Plague: A manual for medical and public health workers. Shanghai Station, China: Weishengshu National Quarantine Service; 1936:334-53.

Davis K.J., S. L. Fritz, M.L. Pitt, S. L. Welkos, P.L. Worsham et al. Pathology of experimental pneumonic plague produced by fraction 1-positive and fraction 1-negative *Yersinia pestis* in African green monkeys (Cercopithecus aethiops). Arch Pathol Lab Med. 1996; 120:156-163.

Dennis DT, Mead PS. Yersinia species, including plague. In Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 7th ed. Philadelphia, PA: Churchill Livingstone; 2009:2943-53.

Doll JM, Zeitz PS, Ettestad P, Bucholtz AL, Davis T, Gage K. Cat-transmitted fatal pneumonic plague in a person who traveled from Colorado to Arizona. Am J Trop Med Hyg 1994;51(1):109-14.

Friedlander, A.M., S. L. Welkos, P.L. Worsham, G.P. Andrews, G. Heath, et al. Relationship between virulence and immunity as revealed in recent studies of the F1 capsule of *Yersinia pestis*. Clin. Infect. Dis. 1995; 21(Suppl. 2):S178-S181.

Huang CH, Huang CY, Chu LW. Pneumonic plague: A report of recovery in a proved case and a note on sulfadiazine prophylaxis. Am J Trop Med Hyg 1948;28(3):361-71.

Inglesby TV, Dennis DT, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, et al. Plague as a biological weapon: medical and public health management. J Am Med Assoc. 2000;283(17):2281-90.

Link VB. A history of plague in the United States of America. [Public Health Monograph No. 26]. Washington DC: US Government Printing Office 1955.

Louisiana Office of Public Health - Infectious Disease Epidemiology Section - Infectious Disease Control Manual. Plague. [Revised 09/25/2004] Available at: http://www.dhh.state.la.us/offices/miscdocs/docs-249/Manual/PlagueManual.pdf. Accessed 08 July 2011.

Meyer K.F., G. Smith, L. Foster, M. Brookman, and M. Sung. Live attenuated Yersinia pestis vaccine: virulent in nonhuman primates, harmless to guinea pigs. J. Infect. Dis. 1974; 129(Suppl):S85-S120.

Munter EJ. Pneumonic plague: Report of a case with recovery. J Am Med Assoc. 1945;128:281-3.

Opulski et al. Pneumonic plague – Arazona, 1992. JAMA 1992; 268:2146-2147.

Simpson WJ. A treatise on plague: dealing with the historical, epidemiological, clinical, therapeutic, and preventive aspects of the disease. Cambridge, England: University Press; 1905. Available at: http://www.books.google.com. Accessed 19 Sep 2011.

Tieh TH, Landauer E, Miyagawa F, Kobayashi G, Okayasu G. Primary pneumonic plague in Mukden, 1946, and report of 39 cases with 3 recoveries. J Infect Dis 1948;82(1):52-8.

Werner SB, Weidmer CE, Nelson BC, Nygaard GS, Goethals RM, Poland JD. Primary plague pneumonia contracted from a domestic cat at South Lake Tahoe, Calif. J Am Med Assoc. 1984;251(7):929-31.

Wong D, Wild MA, Walburger MA, Higgins CL, Callahan M, Czarnecki LA, et al. Primary pneumonic plague contracted from a mountain lion carcass. Clin Infect Dis 2009;49:e33-38.

Wu Lien-Teh. A Treatise on Pneumonic Plague. Geneva, Switzerland: League of Nations. III. Health Organization; 1926:241-95.

8 Appendices

Appendix A
NIAID Independent Pathology Review Summary

Independent Pathology Review

In an effort to apply consistent terminology to the microscopic evaluation of tissues from animals in the Natural History studies for pneumonic plague conducted at Battelle Biomedical Research Center (BBRC), Lovelace Respiratory Research Institute (LRRI) and U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), an independent review of microscopic slides from these animals was conducted.

The general approach to recording microscopic changes resulting from this review of the pathology assessments from these studies was consistent with the perspective and methods provided by Crissman et al 2004, Haschek et al 2010, Shackelford et al 2002, and Wolf and Mann 2005.

The tissue list for microscopic evaluation was different for each study conducted. The only tissues evaluated microscopically in all animals from all studies were lungs and bronchial/tracheobronchial lymph nodes. In addition, the number and/or location of routine sections of lung processed to slide for microscopic evaluation were different for each study (BBRC 617- 2 sections; BBRC 875- 4 sections; LRRI- 7 sections; USAMRIID- 4 sections but not the same locations as in BBRC 875). Each study handled the processing of pulmonary gross lesions in different ways. LRRI for example, always obtained and processed a section of 'lesioned' lung tissue. In BBRC 617, gross lesions in the lung were, in general, processed to slide and evaluated which resulted in the evaluation of from 2 to 7 sections of lungs from animals in this study. Because pneumonic plague in the African Green Monkey appears to be lobar to sublobar in nature, the number and selection of lung tissue presented for microscopic evaluation can result in variability in both character and severity of microscopic findings.

The independent review of microscopic slides from the four natural history studies confirms that there is a common pathology associated with inhaled *Y. pestis* Strain CO92 in African Green Monkeys. Intra-alveolar inflammatory infiltrates (neutrophil and or macrophage) were present in lungs of all animals evaluated from all studies. There were, in several animals, remarkable lobar differences in the presence, severity and/or character of the infiltrates within the same animal. In a single animal, for example, there might be intra-alveolar and intracellular (alveolar macrophages) bacteria, minimal to moderate edema (serous and fibrinous exudates), and a mild to moderate inflammatory infiltrate of primarily macrophages (very few neutrophils) in the left caudal lung lobe whereas the right caudal lung lobe might have large numbers of bacteria widespread in the alveolar spaces, moderate to marked neutrophilic inflammatory infiltration, mild edema, mild to moderate hemorrhage, and fibrinous pleuritis (necrotizing pneumonia). Those areas where edema, macrophages and bacteria predominate appear to represent earlier lesions in the disease process. As the disease progresses, large numbers of neutrophils flood into the affected areas along with hemorrhage and edema which eventually totally efface the normal lung tissue elements. Bacteria are seen in large numbers in the lung and are typically observed in both the alveolar spaces as well as within alveolar macrophages.

Of the lymphoid tissues/organs evaluated, several were affected. Microscopic evaluation of the bronchial/tracheobronchial lymph nodes revealed similar findings across studies. The findings included, bacterial colonization, edema, hemorrhage, inflammatory infiltrates (primarily neutrophil), and lymphoid hyperplasia or depletion. Similar findings were present in mandibular and mediastinal lymph nodes (in studies where these were evaluated). Lymphoid hyperplasia or sinus histocytosis was observed in axillary, inguinal and mesenteric lymph nodes (evaluated in only one study of four animals) however no bacteria or inflammatory infiltrates were noted. One or more of the following changes were observed in

the spleen of all animals evaluated except for one animal from LRRI FY06-126: bacteria, hemorrhage, inflammatory infiltrates (neutrophil) and lymphoid depletion.

Bacteria, edema, hemorrhage, and neutrophilic inflammatory infiltrates were also observed in the connective tissue of the mediastinum in three of the four animals evaluated (USAMRIID F03-09G). This was the only other tissue evaluated which showed microscopic lesions clearly attributable to aerosol exposure of *Y. pestis* Strain CO92 in African Green Monkeys.

In conclusion, independent pathology review of the microscopic slides from the four natural history studies confirms that there is a common pathology associated with lethal infection by inhaled *Y. pestis* Strain CO92 in African Green Monkeys. Post-challenge infection and dissemination occurs quickly in the African Green Monkey. Morphologic changes in the lung appear to begin as lobar to sublobar serous and fibrinous exudates (edema) with intra-alveolar and intracellular (macrophages) bacteria along with increased numbers of alveolar macrophages. These changes observed in the lung transition quickly to diffuse necrotizing pneumonia characterized by alveoli and airways filled with bacteria, inflammation and hemorrhage. There is also dissemination of bacteria to bronchial/tracheobronchial and mediastinal lymph nodes, mediastinal connective tissues and spleen which initiate changes in the tissues such as hemorrhage, inflammation and edema.

A summary of the microscopic findings in the lung and the extensive Interlaboratory Microscopic Finding Incidence Table are found on the following pages of this Appendix.

References:

Crissman JW, Goodman DG, Hildebrandt PK, Maronpot RR, Prater DA, Riley JH, Seaman WJ, Thake DC. (2004). Best practices guideline: toxicologic histopathology. Toxicol Pathol. 32:126-31.

Haschek WA, Rousseaux CG and Wallig MA. (2010). Nomenclature: terminology for morphologic alterations. In: Fundamentals of toxicologic pathology, Second Edition, pp 67-80. Academic Press, San Diego.

Shackelford C, Long G, Wolf J, Okerberg C, Herbert R. Qualitative and quantitative analysis of nonneoplastic lesions in toxicology studies. Toxicol Pathol. 2002 Jan-Feb;30(1):93-6.

Wolf DC and Mann PC. (2005). Confounders in interpreting pathology for safety and risk assessment. Toxicol Appl Pharmacol. 202:302-8.

Summary of Microscopic Findings in the Lungs

Tissue/Finding	Natural History Study				Total
	BBRC 617	BBRC 875	LRRI FY06-126	USAMRIID F03-09G	
Number Examined Lung	10	10	10	4	34
Bacteria	10	10	9	4	33
Edema	10	10	6	3	29
Hemorrhage	8	9	10	4	31
Inflammatory infiltrate, intra-alveolar, macrophage	10	6	10	4	30
Inflammatory infiltrate, intra-alveolar, neutrophil	10	6	10	4	30
Necrosis, multifocal	0	4	0	0	4
Pleura, fibrin	3	8	10	3	24
Pleura, inflammatory infiltrate, macrophage	1	0	0	0	1
Within normal limits	0	0	0	0	0

BBRC = Battelle Biomedical Research Center; LRRI = Lovelace Respiratory Research Institute; USAMRIID = United States Army Medical Research Institute of Infectious Diseases

Interlaboratory Microscopic Finding Incidence Summary

Natural History Studies of Pneumonic Plague following Aerosol Challenge
African Green Monkeys; Male and Female

Incidence of Neoplastic and Non-Neoplastic Microscopic Findings					
	Natural History Study				
Tissue/Observation	BBRC 617	BBRC 875	LRRI FY06-126	USAM RIID F03-09G	Total
No. of Animals:[a]	10	10	10	4[b]	34
Adrenal glands No. Examined:	0	0	0	4	4
Corticomedullary junction, amyloid	–	–	–	1	1
Within normal limits	–	–	–	3	3
Aorta No. Examined:	0	0	0	4	4
Within normal limits	–	–	–	4	4
Bone marrow No. Examined:	0	0	0	4	4
Bacteria	–	–	–	2	2
Myeloid hyperplasia	–	–	–	2	2
Within normal limits	–	–	–	0	0
Brachial plexus No. Examined:	0	0	0	4	4
Within normal limits	–	–	–	4	4
Brain No. Examined:	10	1	10	4	25
Meninges, bacteria	0	1	0	0	1
Within normal limits	10	0	10	4	24
Epididymis No. Examined:	0	0	0	2	2
Within normal limits	–	–	–	2	2
Esophagus No. Examined:	0	0	0	4	4
Adventitia, hemorrhage	–	–	–	1	1
Bacteria	–	–	–	1	1
Inflammatory infiltrate, neutrophil	–	–	–	1	1
Within normal limits	–	–	–	3	3
Eye No. Examined:	0	0	0	4	4
Within normal limits	–	–	–	4	4

Incidence of Neoplastic and Non-Neoplastic Microscopic Findings					
	Natural History Study				
Tissue/Observation	BBRC 617	BBRC 875	LRRI FY06-126	USAMRIID F03-09G	Total
No. of Animals:[a]	10	10	10	4[b]	34
Gallbladder No. Examined:	0	0	0	4	4
Autolysis precludes evaluation	–	–	–	3	3
Within normal limits	–	–	–	1	1
Heart No. Examined:	10	0	0	4	14
Myocardium, fibrosis	1	–	–	1	2
Myocardium, interstitium, cellular infiltrate, lymphoplasmacytic	0	–	–	1	1
Protozoal cyst	1	–	–	0	1
Within normal limits	8	–	–	2	10
Ileocecal junction No. Examined:	0	0	0	4	4
Submucosa, cellular infiltrate, lymphoplasmacytic	–	–	–	1	1
Within normal limits	–	–	–	3	3
Intestine, large, colon No. Examined:	0	0	0	4	4
Tunica muscularis, hemorrhage	–	–	–	1	1
Inflammatory infiltrate, neutrophil	–	–	–	1	1
Within normal limits	–	–	–	3	3
Intestine, small, duodenum No. Examined:	0	0	0	4	4
Within normal limits	–	–	–	4	4
Kidneys No. Examined:	10	0	0	4	14
Glomerulus, fibrin	1	–	–	0	1
Microgranuloma	1	–	–	0	1
Tubule, dilatation	0	–	–	1	1
Tubule, mineral	0	–	–	1	1
Tubulo-interstitial, inflammation, chronic	1	–	–	0	1
Within normal limits	8	–	–	2	10

Incidence of Neoplastic and Non-Neoplastic Microscopic Findings					
	Natural History Study				
Tissue/Observation	BBRC 617	BBRC 875	LRRI FY06-126	USAMRIID F03-09G	Total
No. of Animals:[a]	10	10	10	4[b]	34
Larynx No. Examined:	0	0	0	4	4
Muscularis, myofiber, degeneration	–	–	–	2	2
Skeletal muscle, hemorrhage	–	–	–	1	1
Skeletal muscle, inflammatory infiltrate, neutrophil	–	–	–	1	1
Skeletal muscle, myocyte, degeneration	–	–	–	1	1
Skeletal muscle, protozoal cyst	–	–	–	2	2
Skeletal muscle, cellular infiltrate, lymphoplasmacytic	–	–	–	1	1
Submucosa, cellular infiltrate, lymphoplasmacytic	–	–	–	1	1
Within normal limits	–	–	–	1	1
Lip No. Examined:	0	0	0	4	4
Within normal limits	–	–	–	4	4
Liver No. Examined:	10	0	10	4	24
Bacteria	0	–	1	0	1
Congestion	0	–	1	0	1
Hepatocyte, degeneration, single cell	0	–	0	1	1
Hepatocyte, hydropic change	0	–	7	0	7
Inflammatory infiltrate, neutrophil	0	–	1	0	1
Periportal, cellular infiltrate, lymphoplasmacytic	0	–	0	2	2
Sinusoid, thrombi	0	–	0	1	1
Within normal limits	10	–	2	1	13

Incidence of Neoplastic and Non-Neoplastic Microscopic Findings					
	Natural History Study				
Tissue/Observation	BBRC 617	BBRC 875	LRRI FY06-126	USAM RIID F03-09G	Total
No. of Animals:[a]	10	10	10	4[b]	34
Lung No. Examined:	10	10	10	4	34
Bacteria	10	10	9	4	33
Edema	10	10	6	3	29
Hemorrhage	8	9	10	4	31
Inflammatory infiltrate, intra-alveolar, macrophage	10	6	10	4	30
Inflammatory infiltrate, intra-alveolar, neutrophil	10	6	10	4	30
Necrosis, multifocal	0	4	0	0	4
Pleura, fibrin	3	8	10	3	24
Pleura, inflammatory infiltrate, macrophage	1	0	0	0	1
Within normal limits	0	0	0	0	0
Lymph node, axillary No. Examined:	0	0	0	4	4
Lymphoid hyperplasia	–	–	–	4	4
Within normal limits	–	–	–	0	0
Lymph node, bronchial/ tracheobronchial No. Examined:	10	10	10	4	34
Bacteria	10	10	8	4	32
Edema	3	7	5	4	19
Hemorrhage	8	6	3	0	17
Inflammatory infiltrate, intra-alveolar, neutrophil	8	4	4	3	19
Lymphoid depletion	1	10	2	0	13
Lymphoid hyperplasia	0	0	2	1	3
Within normal limits	0	0	0	0	0
Lymph node, inguinal No. Examined:	0	0	0	4	4
Sinus histiocytosis	–	–	–	1	1
Lymphoid hyperplasia	–	–	–	3	3
Within normal limits	–	–	–	0	0

Incidence of Neoplastic and Non-Neoplastic Microscopic Findings					
	Natural History Study				
Tissue/Observation	BBRC 617	BBRC 875	LRRI FY06-126	USAM RIID F03-09G	Total
No. of Animals:[a]	10	10	10	4[b]	34
Lymph node, mandibular No. Examined:	3	1	0	4	8
Bacteria	3	1	–	0	4
Hemorrhage	2	1	–	0	3
Inflammatory infiltrate, neutrophil	1	0	–	0	1
Lymphoid depletion	0	1	–	0	1
Lymphoid hyperplasia	0	0	–	3	3
Perinodal connective tissue, hemorrhage	0	0	–	1	1
Within normal limits	0	0	–	1	1
Lymph node, mediastinal No. Examined:	10	10	0	4	24
Bacteria	10	9	–	4	23
Edema	0	1	–	4	5
Hemorrhage	5	5	–	1	11
Inflammatory infiltrate, neutrophil	7	1	–	4	12
Lymphoid depletion	0	8	–	0	8
Lymphoid hyperplasia	0	0	–	1	1
Within normal limits	0	1	–	0	1
Lymph node, mesenteric No. Examined:	0	0	0	4	4
Lymphoid hyperplasia	–	–	–	4	4
Within normal limits	–	–	–	0	0
Lymph node, other No. Examined:	0	2	0	0	2
Bacteria	–	1	–	–	1
Hemorrhage	–	1	–	–	1
Lymphoid hyperplasia	–	2	–	–	2
Within normal limits	–	0	–	–	0
Mammary gland and nipple No. Examined:	0	0	0	1	1
Within normal limits	–	–	–	1	1
Mediastinum No. Examined:	0	0	0	4	4
Bacteria	–	–	–	2	2
Connective tissue, edema	–	–	–	3	3
Connective tissue, hemorrhage	–	–	–	2	2
Connective tissue, inflammatory infiltrate, neutrophil	–	–	–	2	2
Within normal limits	–	–	–	1	1

Incidence of Neoplastic and Non-Neoplastic Microscopic Findings					
	Natural History Study				
Tissue/Observation	BBRC 617	BBRC 875	LRRI FY06-126	USAMRIID F03-09G	Total
No. of Animals:[a]	10	10	10	4[b]	34
Muscle, skeletal, quadriceps No. Examined:	0	0	0	4	4
Hemorrhage	–	–	–	1	1
Inflammatory, infiltrate, neutrophil	–	–	–	1	1
Myocyte, degeneration	–	–	–	1	1
Myofiber, protozoal cyst	–	–	–	2	2
Within normal limits	–	–	–	1	1
Nares No. Examined:	0	0	0	4	4
Within normal limits	–	–	–	4	4
Nerve, sciatic No. Examined:	0	0	0	4	4
Within normal limits	–	–	–	4	4
Oropharynx No. Examined:	0	0	0	3	3
Within normal limits	–	–	–	3	3
Ovaries No. Examined:	0	0	0	2	2
Within normal limits	–	–	–	2	2
Oviduct No. Examined:	0	0	0	1	1
Within normal limits	–	–	–	1	1
Pancreas No. Examined:	0	0	0	4	4
Within normal limits	–	–	–	4	4
Parathyroid glands No. Examined:	0	0	0	1	1
Within normal limits	–	–	–	1	1
Pituitary gland No. Examined:	0	0	0	4	4
Within normal limits	–	–	–	4	4
Prostate No. Examined:	0	0	0	2	2
Within normal limits	–	–	–	2	2
Salivary gland, submandibular No. Examined:	0	0	0	4	4
Cellular infiltrate, lymphoplasmacytic	–	–	–	3	3
Within normal limits	–	–	–	1	1

Incidence of Neoplastic and Non-Neoplastic Microscopic Findings					
	Natural History Study				
Tissue/Observation	BBRC 617	BBRC 875	LRRI FY06-126	USAM RIID F03-09G	Total
No. of Animals:[a]	10	10	10	4[b]	34
Skin, haired No. Examined:	0	0	1	4	5
Within normal limits	–	–	1	4	5
Spleen No. Examined:	10	2	10	4	26
Bacteria	9	1	5	3	18
Congestion	0	0	0	1	1
Hemorrhage	4	2	5	0	11
Inflammatory infiltrate, neutrophil	1	0	7	4	12
Lymphoid depletion	4	2	0	0	6
Within normal limits	0	0	1	0	1
Stomach No. Examined:	0	0	1	4	5
Glandular, submucosa, hemorrhage	–	–	1	0	1
Within normal limits	–	–	0	4	4
Testes No. Examined:	0	0	0	2	2
Within normal limits	–	–	–	2	2
Thymus No. Examined:	0	2	0	4	6
Edema	–	2	–	0	2
Hassal's corpuscle, degeneration, cystic	–	0	–	1	1
Hemorrhage	–	2	–	0	2
Lymphoid depletion	–	1	–	0	1
Within normal limits	–	0	–	3	3
Thyroid glands No. Examined:	0	0	0	4	4
Within normal limits	–	–	–	4	4
Tissue, Not Otherwise Specified (NOS) No. Examined:	0	0	1	0	1
Within normal limits	–	–	1	–	1
Tongue No. Examined:	0	0	0	4	4
Cellular infiltrate, lymphoplasmacytic	–	–	–	2	2
Skeletal muscle, protozoal cyst	–	–	–	2	2
Within normal limits	–	–	–	1	1

Incidence of Neoplastic and Non-Neoplastic Microscopic Findings					
	Natural History Study				
Tissue/Observation	BBRC 617	BBRC 875	LRRI FY06-126	USAMRIID F03-09G	Total
No. of Animals:[a]	10	10	10	4[b]	34
Tonsil No. Examined:	0	0	0	4	4
Crypt, bacteria	–	–	–	1	1
Crypt, inflammatory cells, neutrophil	–	–	–	2	2
Crypt, keratin aggregate	–	–	–	3	3
Skeletal muscle, protozoal cyst	–	–	–	1	1
Submucosa, hemorrhage	–	–	–	1	1
Within normal limits	–	–	–	0	0
Trachea No. Examined:	0	0	0	4	4
Skeletal muscle, protozoal cyst	–	–	–	1	1
Skeletal muscle, cellular infiltrate, lymphoplasmacytic	–	–	–	1	1
Submucosa, bacteria	–	–	–	1	1
Submucosa, inflammatory infiltrate, neutrophil	–	–	–	1	1
Within normal limits	–	–	–	3	3
Ureter No. Examined:	0	0	0	1	1
Within normal limits	–	–	–	1	1
Urinary bladder No. Examined:	0	0	0	4	4
Within normal limits	–	–	–	4	4
Uterus No. Examined:	0	0	0	2	2
Within normal limits	–	–	–	2	2
Vagina No. Examined:	0	0	0	1	1
Mucosa, cellular infiltrate, lymphoplasmacytic	–	–	–	1	1
Within normal limits	–	–	–	0	0
Vein, large (jugular) No. Examined:	0	0	0	2	2
Endothelium, ulceration	–	–	–	1	1
Thrombus, organizing	–	–	–	1	1
Within normal limits	–	–	–	0	0

Appendix B
NIAID Independent Radiology Review Summary

AGM Model of Pneumonic Plague

In an effort to apply consistent terminology to the radiographic interpretation of radiographs from animals in the Natural History studies for pneumonic plague conducted at Lovelace Respiratory Research Institute (LRRI) and U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), an independent review of radiographs from these animals was conducted.

The independent review of radiographs from the two natural history studies confirms that there is a common radiographic pulmonary appearance and progression of the severity of changes over time associated with inhaled *Y. pestis* Strain CO92 in African Green Monkeys.

A summary of the radiographic findings in the lung is found in Table 1.

Table 1 Summary of Radiographic Findings in the Lungs (Natural History Studies)

	LRRI FY06-126			**USAMRIID F03-09G[a]**		
Days Post Challenge	Baseline	Day 3	Day 4	Baseline	80-83hr	Terminal
Lung Number Examined	9	9	9	4	4	4
Normal	7	2	1	4	0	0
Pulmonary infiltrate						
Mild	2	4	1	0	3	0
Moderate	0	2	0	0	1	4
Severe (marked)	0	1	7	0	0	0

Hr = hours; LRRI = Lovelace Respiratory Research Institute; USAMRIID = United States Army Medical Research Institute of Infectious Diseases

[a]The independent review radiologist was only provided one un-dated radiograph from Animal V521 so the results from this animal are not included in this table. The independent review radiologist was provided two un-dated radiographs from Animal V569. These were tabulated in the table as being from the 80-83 hour and terminal timepoints.