**Supplemental Material**

**Physiological responses to multiple low-doses of *Bacillus anthracis* spores in the rabbit model of inhalation anthrax**

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Table S1. Summary table for single and multiple dose study data

**Single Dose (**[**1**](#_ENREF_1)**) Multiple Dose**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Order of Magnitude Dose for Comparison** | **Mean Dose Group Inhaled Dose (GSD)**  **(Colony Forming Units (CFU))** | **Rabbit Identification** | **Survival or Day of Death** | **Rabbit**  **Identification** | **Individual Rabbit Accumulated Dose** | **Survival or Day of Death after First Dose** |
|  | 2.06 x 103 | L23217 | Survived | 13 | 5.78 × 103 | Survived |
|  | (3.42 x 102) | L23230 | Survived | 34 | 4.76 × 103 | Survived |
| 103 |  | L23228 | Survived | 25 | 4.19 × 103 | Survived |
|  |  | L23227 | Survived | 15 | 4.76 × 103 | Survived |
|  |  | L23229 | Survived | 30 | 4.07 × 103 | Survived |
|  |  |  |  | 28 | 3.51 × 103 | Survived |
|  |  |  |  | 19 | 3.48 × 103 | Survived |
|  | 2.54 x 104 | L23235 | 11 | 14 | 1.11 × 104 | Survived |
|  | (5.21 x 103) | L23205 | Survived | 11 | 1.68 × 104 | Survived |
|  |  | L23225 | 4 | 2 | 1.86 × 104 | 17.9 |
|  |  | L23231 | Survived | 8 | 2.10 × 104 | Survived |
| 104 |  | L23207 | Survived | 12 | 1.95 × 104 | Survived |
|  |  |  |  | 18 | 1.85 × 104 | Survived |
|  |  |  |  | 32 | 2.83 × 104 | Survived |
|  |  |  |  | 6 | 5.77 × 104 | 10.9 |
|  |  |  |  | 33 | 9.75 × 104 | 12.7 |
|  | 2.75 x 105 | L23201 | 4 | 27 | 1.51 × 105 | 20.8 |
|  | (7.41 x 104) | L23234 | 6 | 31 | 1.37 × 105 | 14.7 |
| 105 |  | L23212 | Survived | 39 | 2.16 × 105 | Survived |
|  |  | L23200 | 3 | 21 | 1.98 × 105 | Survived |
|  |  | L23214 | 6 | 38 | 1.91 × 105 | Survived |
|  | 8.27 x 106 | L23204 | 4 | N/A |  |  |
|  | (1.69 x 106) | L23203 | 5 |  |  |  |
| 106 |  | L23213 | 3 |  |  |  |
|  |  | L23221 | 2 |  |  |  |
|  |  | L23232 | 4 |  |  |  |

GSD geometric standard deviation

N/A Not applicable, no testing performed at this dose

Table S2. Summary of individual gross and microscopic observations

| **Dose Group** | **Animal Number/ Death Statusa** | **Gross Findings** | **Microscopic Findings** |
| --- | --- | --- | --- |
| Control | 40/FS |  | Lung: Unremarkable. |
| 7/FS |  | Lung: Unremarkable. |
| 5/FS |  | Lung: Perivascular eosinophils, minimal. |
| 9/FS |  | Lung: Perivascular eosinophils, minimal. |
| 37/FS |  | Lung: Unremarkable. |
| 100 CFU | 13/FS |  | Lung: Perivascular eosinophils, minimal. |
| 34/FS |  | Lung: Foreign body, mild.  Lung: Multinucleated giant cells, mild. |
| 25/FS |  | Lung: Unremarkable. |
| 15/FS |  | Lung: Perivascular eosinophils, minimal. |
| 30/FS |  | Lung: Unremarkable. |
| 28/FS |  | Lung: Perivascular eosinophils, mild. |
| 19/FS |  | Lung: Unremarkable. |
| 1000 CFU | 14/FS |  | Lung: Perivascular eosinophils, minimal. |
| 11/FS |  | Lung: Perivascular eosinophils, minimal. |
| 2/FD |  | Lung: Hemorrhage, minimal.  Lung: Inflammation, suppurative, minimal.  Lung: Bacteria, minimal. |
| 8/FS |  | Unremarkable. |
| 12/FS | Skin: Laceration(s), hindlimb, red, left hindlimb, 40 x 20 mm | Lung: Foreign body, minimal.  Lung: Multinucleated giant cells, mild.  Skin: Inflammation, necrosuppurative, marked. |
| 306 (18)/FS |  | Lung: Unremarkable. |
| 307 (32)/FS |  | Lung: Perivascular eosinophils, minimal. |

FD = Found Dead, FS = Final Phase Sacrifice, mm = millimeters

|  |  |  |  |
| --- | --- | --- | --- |
| **Dose Number** | **Animal Number/ Death Statusa** | **Gross Findings** | **Microscopic Findings** |
| 10,000 CFU | 6/FD | Cecum: Accumulation (gas)*.* Samples of cecum, colon, jejunum, and appendix were collected to confirm lesion. | Cecum: Edema, mild.  Cecum: Edema, hemorrhage and necrosis.  Cecum: Hemorrhage, moderate.  Cecum: Necrosis, moderate.  Lung: Perivascular eosinophils, minimal. |
| 33/FD | Lymph Node, Mediastinal: Enlarged, dark, 3x. | Lung: Bacteria, mild.  Lung: Hemorrhage, minimal.  Lung: Inflammation, suppurative, mild.  Lung: Perivascular eosinophils, minimal.  Lymph Node, Mediastinal: Bacteria, Marked.  Lymph Node, Mediastinal: Edema, fibrin, mild.  Lymph Node, Mediastinal: Hemorrhage, minimal.  Lymph Node, Mediastinal: Necrosis/depletion, lymphoid, marked. |
| 27FD | Appendix: Foci, multiple, red, up to 2 x 2  mm. | Appendix: Hemorrhage, mild.  Appendix: Necrosis/depletion, lymphoid, moderate.  Appendix: Infiltration cellular, macrophages, moderate.  Appendix: Note: hemorrhage and necrosis.  Lung: Bacteria, minimal.  Lung: Inflammation, suppurative, minimal.  Lung: Perivascular eosinophils, minimal. |
| 31/FD |  | Lung: Bacteria, mild.  Lung: Inflammation, suppurative, minimal. |
| 39/FS |  | Lung: Foreign body, minimal.  Lung: Multinucleated giant cells, minimal. |
| 21/FS |  | Lung: Unremarkable. |
| 38/FS | Lung: Discoloration(s), apical lobe, pale, firm.  Skin: Laceration(s), abdominal, red,  20 x 15 mm. | Lung: Foreign body, moderate.  Lung: Granuloma/pyrogranuloma, moderate.  Lung: Perivascular eosinophils, minimal.  Skin: Inflammation, necrosuppurative, moderate.  Skin: Thrombosis, artery, mild. |

FD = Found Dead, FS = Final Phase Sacrifice, mm = millimeters

**TABLE S3.** **Bacteremia measurements in colony forming units (CFU) for animals in the single dose study Taft et al. (**[**1**](#_ENREF_1)**) with measured positive bacteremia results**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *Dose* | *Animal* | *Day 1* | *Day 2* | *Day 3* | *Day 4 and Latera* |
| 2.54 x 104 | L23235 | ND | ND | ND | ND on Day 7  TTD = 11 1.7 x 106 |
| L23225 | ND | 4.53 x 103 | <2.5 x 103  (<LLOQ) | TTD = 4 8.03 x 105 |
| 2.75 x 105 | L23201 | ND | <2.5 x 103  (<LLOQ) | 9.73 x 105 | TTD = 4 9.8 x 105 |
| L23234 | ND | ND | <2.5 x 103  (<LLOQ) | TTD = 6 5.43 x 106 |
| L23200 | <2.5 x 103  (<LLOQ) | <2.5 x 103  (<LLOQ) | TDD = 3 <2.5 x 103 |  |
| L23214 | ND | 8.23 x 103 | ND using qualitative test | TTD = 6 ND |
| 8.27 x 106 | L23204 | <2.5 x 103  (<LLOQ) | <2.5 x 103  (<LLOQ) | 2.8 x 103 | TTD = 4 1.15 x 105 |
| L23203 | ND | <2.5 x 103  (<LLOQ) | <2.5 x 103  (<LLOQ) | TTD = 5 3.03 x 107 |
| L23213 | ND | <2.5 x 103  (<LLOQ) | TTD = 3 1.31 x 105 |  |
| L23221 | NS | TTD = 2 2.58 x 106 |  |  |
| L23232 | <2.5 x 103  (<LLOQ) | ND | 2.77 x 103 | TTD = 4 ND using qualitative test |

a Bacteremia measurements conducted on Study Days 1, 2, 3, 7, 14, and 21

LLOQ – lower level of quantification of 2.5 x 103 CFU

ND – not detected in sample

NS – no sample tested

TTD – time to death from challenge day

TABLE S4. Comparison of multiple dose study days between last blood draw and time to death with Comer et al. ([2](#_ENREF_2)), Yee et al. ([3](#_ENREF_3)), and Taft et al. ([1](#_ENREF_1)) bacteremic days prior to death

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Animal Number** | **Time to Death (TTD)** | **Days Between Last Blood Draw and Death** | **Potential Capture Using**  **Comer et al. (**[**2**](#_ENREF_2)**) Rangeb** | Potential Capture Using Yee et al. ([3](#_ENREF_3)) Rangec | Potential Capture Using Taft et al. ([1](#_ENREF_1)) Ranged |
| 2 | 17.9 | 1.9 | Yes | No | Yes |
| 6 | 10.9 | 1.9 | Yes | No | Yes |
| 33 | 12.7 | 1.7 | Yes | Yes | No |
| 27  31 | 20.8  14.7 | 2.8  3.7 | Yes  No | No  No | Yes  Yes |

a Blood Draw Days: 2, 4, 9, 11, 16, 18, 23, 25, 30, 32, 37, 39

b Range of Calculated Study Days Between Detectable Bacteremia and Death: 0.58 to 3.64 Days, Mean of 1.99 Days

c Range of Calculated Study Days Between Detectable Bacteremia and Death: 0.33 to 1.75 Days, Mean of 0.91 Days

d Range of Calculated Study Days Between Detectable Bacteremia and Death: 1.9 to 3.9 Days, Mean of 2.8 Days

TABLE S5. Protective antigen measurements for all animals in the single dose study with positive protective antigen results

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *Dose* | *Animal* | *Day 1* | *Day 2* | *Day 3* | *Day 4 and Latera* |
| 2.54 x 104 | L23235 | ND | ND | ND | ND on Day 7  TTD = 11  NS on TD |
| L23225 | ND | ND | 51 | TTD = 4  33,000 (>ULOQ) |
| 2.75 x 105 | L23201 | ND | 65 | 471 | TTD = 4  NS on TD |
| L23234 | ND | ND | 20 | TTD = 6  NS on TD |
| L23200 | ND | ND | TTD = 3  NS on TD |  |
| L23214 | ND | 28.6 | NS | TTD = 6  NS on TD |
| 8.27 x 106 | L23204 | ND | ND | 9.6 | TTD = 4  25,000 (>ULOQ) |
| L23203 | ND | 2.8 | 1.6 | TTD = 5  NS |
| L23213 | ND | 125 | TTD = 3 383 |  |
| L23221 | ND | TTD = 2  NS on TD |  |  |
| L23232 | ND | ND | 2.1 | TTD = 4  NS on TD |

a Protective antigen measurements post-exposure conducted on Study Days 1, 2, 3, 7, 14, and 21

ND – not detected in sample, level of detection of 2.0 ng/mL and lower limit of quantification (LLOQ) (4.9 ng/mL)

NS – no sample tested

TD – terminal day

TTD – time to death from challenge day

ULOQ – upper level of quantification (10,000 ng/mL)

**Bibliography**

1. **Taft SC, Nichols TL, Hines SA, Barnewall RE, Stark GV, Comer JE.** 2017. Physiological responses to a single low-dose of *Bacillus anthracis* spores in the rabbit model of inhalational anthrax. Submitted to Infection and Immunity as First Article of Two Companion Articles

2. **Comer JE, Ray BD, Henning LN, Stark GV, Barnewall RE, Mott JM, Meister GT.** 2012. Characterization of a therapeutic model of inhalational anthrax using an increase in body temperature in New Zealand white rabbits as a trigger for treatment. Clinical and Vaccine Immunology : CVI **19:**1517-1525.

3. **Yee SB, Hatkin JM, Dyer DN, Orr SA, Pitt MLM.** 2010. Aerosolized *Bacillus anthracis* infection in New Zealand White Rabbits: Natural history and intravenous levofloxacin treatment. Comp. Med. **60:**461-468.