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Immunogenicity and Safety of Four Different Dosing Regimens of Anthrax Vaccine Adsorbed for Post-Exposure Prophylaxis for Anthrax in Adults

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Abstract

Background—Strategies to implement post exposure prophylaxis (PEP) in case of an anthrax bioterror event are needed. To increase the number of doses of vaccine available we evaluated reducing the amount of vaccine administered at each of the vaccinations, and reducing the number of doses administered.

Methods—Healthy male and non-pregnant female subjects between the ages of 18 and 65 were enrolled and randomized 1:1:1:1 to one of four study arms to receive 0.5 mL (standard dose) of vaccine subcutaneously (SQ) at: A) days 0, 14; B) days 0 and 28; C) days 0, 14, and 28; or D) 0.25 ml at days 0, 14, and 28. A booster was provided on day 180. Safety was assessed after each dose. Blood was obtained on days 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 84, 100, 180, and 201 and both Toxin Neutralizing antibody and anti-PA IgG antibody measured.

Results—Almost all subjects developed some local reactions with 46% to 64% reported to be of moderate severity and 3.3% severe during the primary series. Vaccine groups that included a day 14 dose induced a 4 fold antibody rise in more subjects on days 21, 28 and 35 than the arm without a day 14 dose. However, schedules with a full day 28 dose induced higher peak levels of

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Conflicts of interest: None to report

antibody that persisted longer. The half dose regimen did not induce antibody as well as the full dose study arms.

Conclusion—Depending on the extent of the outbreak, effectiveness of antibiotics and availability of vaccine, the full dose 0, 28 or 0, 14, 28 schedules may have advantages.

Keywords

Anthrax; vaccine; post exposure prophylaxis

Introduction

Anthrax is an acute infectious disease caused by the spore-forming bacterium *Bacillus anthracis* (*B. anthracis*). Cutaneous anthrax is the most commonly reported form in humans (greater than 95% of all cases) and occurs when the bacterium enters a cut or abrasion on the skin. Gastrointestinal anthrax usually occurs after ingestion of meat contaminated with anthrax spores. Inhalational anthrax is the most lethal form of anthrax[1] and occurs after exposure to aerosolized spores[2]. A major concern is the potential to weaponize the spores and use it as an agent of bioterrorism.

Since the majority of individuals are not vaccinated against anthrax, strategies to implement post exposure prophylaxis (PEP) in case of a bioterror event are needed. Although anthrax vaccine adsorbed (AVA, BioThrax[®]) is currently only approved for pre-exposure prophylaxis, a 60-day course of antibiotics in conjunction with three doses of BioThrax[®] two weeks apart are recommended by the Advisory Committee on Immunization Practices (ACIP) for PEP[3]. Although antibiotics are effective when administered prior to or immediately after spore exposure, residual spores can germinate and release toxin after discontinuation of antibiotics, causing disease and death[4–6]. To be highly effective, antimicrobial therapy must be initiated as soon as possible after infection since efficacy diminishes rapidly as toxemia progresses. However, even if mass distribution of antimicrobials can be aggressively completed within six days of the initial exposure, it is estimated that only approximately 70% of cases can be prevented[7]. For these reasons, it is critical to combine antibiotics with vaccination in the post-exposure setting.

The current target dosing schedule for PEP is a full dose (0.5 mL) administered on day 0, 14, and 28. In the event of a large anthrax outbreak, it is possible that there will be an insufficient number of doses available to vaccinate the entire exposed population. Strategies to increase the number of doses available include reducing the amount of vaccine administered at each of the vaccinations, or reduce the number of doses administered. Policy makers need data on the immunogenicity of alternative dosing regimens. The purpose of this protocol was to generate these data. We compared the antibody response to the standard three-dose schedule to the antibody response of two-dose vaccination regimens (days 0 and 14, and days 0 and 28) using full dose (0.5 mL) BioThrax[®] and to the antibody response of a three-dose regimen (days 0, 14, and 28) using half the dose (0.25 mL) of BioThrax[®] in adults. Since it may become necessary to ensure longer term protection for individuals who received a PEP regimen, a 6-month boost was also administered to explore the effect of a booster dose when administered following the different PEP regimens.

Methods

Subjects

Healthy male and non-pregnant female subjects between the ages of 18 and 65 were recruited at 4 sites within the USA. Subjects were excluded if they had a prior anthrax immunization, were allergic to any vaccine component, had received immunosuppressive therapy, blood products or immunoglobulin within 3 months, had a history of Guillain-Barre Syndrome, a malignancy, diabetes requiring insulin, significant cardiovascular, pulmonary, renal, autoimmune, inflammatory, vasculitic, rheumatic, neurologic or liver disease. Those with significant psychiatric disease or taking more than one antidepressant or selected psychiatric drugs were also excluded. Subjects were screened for HIV, hepatitis B surface antigen, and antibody to hepatitis C virus. For females of childbearing potential, a serum pregnancy test was performed. These screening tests had to be negative for subjects to be eligible for participation in the study. Serum chemistries were also obtained prior to enrollment and were required to be normal or near normal. Tattoos that obscured the vaccination site were not allowed. Full inclusion and exclusion criteria are available at [Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01641991) NCT01641991.

Vaccine

Anthrax vaccine adsorbed (AVA, BioThrax[®]) was supplied by the Centers for Disease Control and Prevention in 5 mL multi-dose vials. It was made from cell-free filtrates of microaerophilic cultures of an avirulent, nonencapsulated strain of *Bacillus anthracis*. The final product contains culture fluid proteins including the *B. anthracis* protective antigen (PA) and 1.2 mg/mL aluminum, added as aluminum hydroxide in 0.85% sodium chloride.

Study design

This was a randomized, open-label immunogenicity and safety study to evaluate four dosing regimens of BioThrax[®] for PEP for anthrax. Subjects were enrolled and randomized 1:1:1:1 to one of four study arms to receive 0.5 mL (standard dose) of vaccine subcutaneously (SQ) at: A) days 0, 14; B) days 0 and 28; C) days 0,14, and 28; or D) 0.25 ml at days 0,14, and 28. These vaccinations are referred to as the primary series. Enrollment was stratified by gender, with approximately equal numbers of males and females enrolled into each dosing regimen. Subjects were followed for approximately 201 days. Blood was obtained on days 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 84, 100, 180 and 201 and anthrax antibody measured. All subjects received a 0.5 mL dose intramuscularly (IM) at approximately 6 months (booster dose). Systemic and local reactions were collected with the use of a memory aid for at least 8 days (days 0 – 7) following each vaccination. Unsolicited adverse events were collected at every visit up to 28 days post last vaccination with the primary series and then again after the 6 month boost until the day 201 visit. Serious adverse events were collected throughout the study period

Antibody assays

Serum samples were evaluated for levels of anti-anthrax antibodies in both the Toxin Neutralization Activity (TNA) Assay and the anti-PA IgG Enzyme Linked Immunosorbent Assay (ELISA).

TNA Assay—The TNA Assay measures the levels of anthrax lethal toxin neutralizing antibody using an in vitro cytotoxicity assay. The assay was originally validated at the CDC, but was then transferred and validated at Battelle, where the testing of these serum samples occurred[8, 9]. Briefly, microtiter cell plates were seeded with J774A.1 cells and allowed to adhere. In separate microplates a mixture of recombinant protective antigen (rPA, List Biological Laboratories, Inc., Campbell, California, Cat. No. 171B) and recombinant lethal factor (rLF, List Biological Laboratories, Inc., Campbell, California Cat. No. 172B) was added to serial dilutions of the test samples and controls and incubated prior to transfer to the cell plate. The final concentration of rPA was 0.05 µg/mL and the final concentration of rLF was 0.04 µg/mL. MTT was then added to the cell plates to allow viable cells to reduce the MTT dye. The OD values for each plate were read on a BioTek microplate reader at a wavelength of 570 nm using a 690 nm reference wavelength. The TNA SAS program[8] was used to fit the 7-point serial dilutions of the reference serum standard and test sample serum OD values to a four parameter logistic-log (4PL) function, which is in turn was used to calculate the reportable values (ED₅₀ and NF₅₀). The assay endpoints are the Effective Dilution 50 (ED₅₀) and the Neutralization Factor 50 (NF₅₀). The ED₅₀ is the reciprocal of the dilution of a serum sample that results in 50% neutralization of anthrax lethal toxin. The ED₅₀ is determined as the reciprocal of the dilution corresponding to the inflection point ('c' parameter) of the 4-parameter logistic log fit of the curve. The NF₅₀ is the ED₅₀ of the test sample divided by the ED₅₀ of the reference standard. The NF₅₀ Lower Limit of Quantification (LLOQ) was 0.064[10]. The TNA reference standard was the human serum AVR801 (BEI Resources).

ELISA Assay—The anti-PA IgG ELISA measures the quantity of serum anti-PA IgG antibodies. The ELISA was originally validated at the CDC, but was transferred and validated at Battelle, where the testing of these serum samples occurred[11]. Briefly, microtiter plates were coated with 1 µg/mL rPA (List Biological Laboratories, Campbell, California, Catalog Number 171B). Test samples, anti-PA IgG reference standard serum, and positive control sera were then added to the microtiter plate. After washing, the bound anti-PA antibodies were detected by a species-specific anti-gamma chain IgG – horseradish peroxidase (HRPO) conjugate followed by addition of a peroxidase substrate. The optical density (OD) values for each plate were read on a microplate reader at a wavelength of 405 nm using a 490 nm reference wavelength. The anti-PA IgG concentration was determined by taking the average of the acceptable concentrations from the 8-point dilution of the test sample back-calculated from the standard curve. Results are reported in µg/mL of anti-PA IgG. The ELISA LLOQ was 9.27 µg/mL.

Statistical analysis

This study was not designed to test a formal null hypothesis. Hypothesis tests between study arms were carried out in an exploratory fashion. Safety outcome measures were described

using frequency, proportion, and 95% two-sided exact confidence intervals (95% CI). Rates were compared using a Fisher's Exact test. For the immunogenicity analysis, if values were below the LLOQ of the respective assay, LLOQ/2 was imputed. The immune response was analyzed as both binary and continuous variables. When analyzed as a binary outcome, proportions of subjects with 4-fold increase from baseline together with their 95% CI were calculated and compared using a Fisher's Exact test. The geometric mean titer or concentration (GMT or GMC) together with its 95% confidence interval (CI) was calculated for each arm and study day. In addition, the peak titer for each subject between days 0–100 was determined. The geometric mean of the peak titer was used to compare the response magnitude between study arms using a t-test. Supplemental immunogenicity analyses (logistic regression and Analysis of Covariance [ANCOVA]) were carried out to evaluate pairwise treatment effects for discrete and continuous outcomes after adjusting for gender by adding a gender covariate to the statistical model. All tests were two-sided and significance was evaluated at an individual alpha level of 0.05.

For the immunogenicity analysis the intention to treat (ITT, n=328) analysis population included all subjects but 3 for whom the sera were not kept at the specified temperature. All subjects received the treatment that was assigned per the randomization process. The per protocol (PP) analysis population for days 0-day 100 (n=245) included subjects who met all the inclusion and exclusion criteria, received all doses in the primary series, completed all scheduled visits up to and including the day 100 visit in-window, and who contributed both pre- and post-vaccination blood samples for testing for which valid test results were reported. The PP analysis population for days 180-day 201 (n=224) excluded 21 additional subjects due to early termination and out-of-window visits.

Results

Three hundred twenty-eight volunteers were enrolled into this multi-center clinical trial from July 2012 until June 2013. Most subjects were White/Caucasian with a similar distribution by gender, race and ethnicity and an average age between 34.6 and 36.7 years for each study arm (Table 1). Of the 328 subjects enrolled, 278 (84.8%) subjects were followed through the final scheduled visit and 50 (15.2%) subjects were withdrawn from the study including 9 from study arm A, 8 from arm B, 17 from arm C and 17 from arm D (Fig. 1).

Safety

The analysis population (n=269) for the safety data included all enrolled and vaccinated subjects except those at one site who received at least one dose of vaccine in the fatty tissue over the triceps in the outer aspect of the upper arm (instead of the specified site, inferior deltoid). The administration error artificially increased the local reactogenicity for these subjects as detailed below (these subjects were included in the immunogenicity analysis).

Two hundred sixty-one subjects (97%) reported experiencing some local reactogenicity during the primary series (Table 2). Moderate reactions were common and occurred in 46% to 64% in subjects following any of the primary vaccinations. Eleven (4.1%) subjects experienced severe local reactions (9 subjects during the primary series and 2 subjects after

the booster dose). Of these, 8 subjects experienced severe redness and/or swelling only, and 3 experienced severe arm motion limitation, pain, tenderness or itchiness at the injection site. Tenderness was the most common local reaction in all study arms. When comparing local reactions by maximum severity across the primary vaccination series, no differences between arms were observed. However, following the booster dose, Arm A (day 0, 14, 180) had a significantly higher rate of subjects (55%) with moderate or severe local reactions compared to subjects in Arm B (28.3%) and C (33.9%) who received vaccinations at days 0, 28, and 0, 14, 28 respectively. No significant difference was observed between the day 0, 14, 28 half dose (Arm D) and full dose arm (Arm C).

Systemic reactions were less common with 56.7% to 67.2% of subjects in each study arm developing a systemic reaction during the primary series. Two subjects (1.0%), one of them after the booster dose and one after dose 3, experienced severe systemic reactions (one subject with fatigue and one with elevated oral temperature [102.5°F]). Muscle aches were the most common reported solicited systemic symptom. When comparing systemic reactions by maximum severity across the primary vaccination series, no differences between arms were observed. However, following the booster dose, Arm A (day 0, 14, 180) had a significantly higher rate of subjects (20%) with moderate or severe systemic reactions compared to subjects in Arm B (5.0%) who received vaccinations at days 0, 28. No significant difference was observed between the day 0, 14, 28 half dose (Arm D) and full dose arm (Arm C). A total of 926 unsolicited adverse events were reported by 260 subjects (96.7% of 269 subjects). Of these, 3 events in 3 subjects were SAEs (all were assessed as unrelated to vaccine). Six hundred ten non-serious adverse events, reported by 232 subjects (86.2%), were considered related to study product. Of these 610 AEs, 608 were graded as mild or moderate in severity and 2 events (hypertension and tenderness) were graded as severe. Subcutaneous nodules at the injection site accounted for 388 (42.0%) of 923 unsolicited non-serious adverse events reported in 201 subjects (74.7% of 269 subjects in the safety population), of which all were reported as related to study product. Of these 388 nodule events, 344 resolved during the study and the remaining 44 nodules were considered ongoing but stable at the end of follow-up. The rate of nodules at the injection site was not significantly different between any of the four study arms.

Comparison between excluded subjects receiving the vaccine in the triceps (n=59) with those that received the vaccine in the deltoid (n=269) showed the triceps group had significantly higher incidents of injection site redness (120 mm or greater, 88.1% [95% CI: 77.1, 95.1%]), swelling (120 mm or greater, 67.8% [95% CI 54.4, 79.4%]), and itchiness 72.9 % [95% CI: 59.7, 83.6%]) compared to 56.5% [95% CI: 50.4, 62.5%], 47.6% [95% CI: 41.5, 53.7%], and 55% [95% CI: 48.9, 61.1%]), for the deltoid group. Among systemic reactions, the rate of fatigue was significantly lower, 32.2% [95% CI: 20.6, 45.6%] for the triceps group compared to 49.4% [95% CI: 43.3, 55.6%] for the deltoid group.

Immunogenicity

Serum samples were evaluated for levels of anti-anthrax antibodies using both the TNA and the ELISA anti-PA IgG antibody assays. There was a high positive correlation between assays irrespective of study arm by day 21 (Pearson Correlation 0.78). The TNA assay is

highlighted in the comparisons below. Analysis results for ITT and PP populations resulted in overall similar findings, also when adjusted for gender (supplemental analysis, not shown). Thus, unadjusted PP population results are reported and discrepancies with the ITT populations are highlighted.

The antibody response differed significantly by the dosing regimen for TNA NF₅₀ antibody (Table 3, Fig. 2A) and anti-PA IgG measured by ELISA (Table 4, Fig. 2B). One dose of vaccine was not sufficient to induce a 4 fold antibody response in either assay in the majority of subjects but primed for a boost with the second dose. Among subjects receiving the full dose of vaccine, a 4 fold antibody response was detected earlier in both assays for the dosing schedules that included a day 14 dose than the arm that did not. For example, at early time points, subjects receiving a full dose of BioThrax[®] at days 0 and 14 (Arm A and C) had a higher proportion of subjects with a 4 fold rise in TNA NF₅₀ antibody over baseline (38.8% and 32.2% at day 21, 94.0%, and 94.9% at day 28, and 97.0 and 100% at day 35 for Arms A and C, respectively) compared to Arm B (20.3%, 22.0%, 54.2% at days 21, 28, and 35, respectively). Subjects receiving the half dose at days 0, 14, and 28 (Arm D) achieved a 4-fold rise in TNA NF₅₀ antibody over baseline in 20.0%, 76.7%, and 88.3% of subjects at days 21, 28 and 35, respectively. All rates were significantly lower than Arm C for both assays except for the day 14 comparison for TNA NF₅₀ antibody.

By day 56 (TNA NF₅₀) or day 49 (anti-PA IgG) and beyond more subjects in study Arm B and C had 4 fold rises than Arm A. Statistical significance for pairwise comparisons is summarized in Tables 3 and 4.

The day 0, 14 schedule achieved its peak GMT at day 28 (TNA NF₅₀) or day 35 (anti-PA IgG), while the other three arms obtained their peak GMTs at day 42 (Fig. 2A, 2B). The day 0, 28 schedule induced the highest TNA NF₅₀ geometric mean of the peak titer (GMPT=2.99 [95% CI: 2.34, 3.81]) and highest anti-PA IgG titer (GMPT=265.1 [95% CI: 214.1, 328.2]) which were statistically significantly higher compared to all other arms. The three half dose regimen did not induce antibody as well as the three full dose study arm. For example, for TNA NF₅₀ antibody the GMPT was 1.00 [95% CI: 0.76, 1.32] versus GMPT=1.48 [95% CI: 1.22, 1.81]. For both assays, this difference was statistically significant for the PP population but not the ITT population. Subjects belonging to Arm A with a full dose at days 0 and 14 had the lowest peak titer in both assays. In the TNA NF₅₀ assay the GMPT (0.74 [95% CI: 0.59, 0.94]) was statistically significantly lower compared to all other full dose arms. Similar differences were detected for ELISA antibody responses (Fig. 2B).

Antibody titers waned over time but were boosted by the dose administered on day 180 (Table 3, Table 4, Fig. 2A, 2B). At day 180, subjects in study Arm A had a significantly lower proportion of subjects with a 4-fold increase in TNA NF₅₀ titer from baseline (15.3%) compared to all other arms (Arm B: 42.6%, Arm C: 66.1%, and Arm D: 54.5%). This was also true for anti-PA IgG antibody. The difference between Arms C and B was also statistically significant but only for TNA NF₅₀ antibody. A similar pattern was observed when GMTs were compared (Fig. 2A, 2B). At day 201, after receiving the booster dose, all subjects in all study arms had a 4-fold or greater increase in TNA NF₅₀ titer from baseline

and all but one had a 4-fold or greater increase in anti-PA IgG. Antibody levels were higher than the peak response after the 2 or 3 dose priming series (Fig. 2A, 2B). Subjects in Arm A developed the highest antibody levels in both assays; significantly higher than Arm C despite having the lowest titers before the boost.

Discussion

As expected[3], the vaccine was highly reactogenic; almost all subjects developed local reactions with 46% to 64% reported to be of moderate severity and 3.3% severe during the primary series. There were no statistically significant differences in maximum severity of local reactions (moderate or severe) by study arm during the primary series. However, moderate or severe local reactions following the booster dose at day 180 were significantly higher in Arm A (day 0, 14, 180) than the other full dose arms. Systemic reactions were less common with a frequency of 56.7% to 67.2%. Nodules, an unusual event not seen following most other vaccines, accounted for 388 (42.0%) of the unsolicited non-serious adverse events and were reported in 201 subjects (74.7%). Of these 388 nodule events, 344 resolved during the study and the remaining 44 nodule events were considered ongoing but stable at the end of the study.

The antibody response differed significantly by the dosing regimen. Schedules that included a day 14 dose induced an antibody response (both TNA NF₅₀ and anti-PA IgG) earlier than the arm without a day 14 dose. However, schedules with a full day 28 dose induced higher peak levels of antibody that persisted longer. The half dose regimen did not induce antibody as well as the full dose study arms. The day 0, 28 schedule induced the highest peak levels of antibody following the initial schedule. Thus, the advantage of the two study arms with a 14-day full dose is the percent of subjects with a 4-fold rise at days 21, 28 and 35. The disadvantage of the day 0, 14 schedule was that this study arm had significantly lower peak titers. Further, from day 49 (anti-PA IgG) or day 56 (TNA NF₅₀) until the time of the booster dose the percent of subjects with 4-fold rises was also statistically significantly less than the percent of subjects in the study arms receiving a full dose at day 28. Therefore, during an outbreak the advantage of a rapid, early response would need to be weighed against the magnitude of the later response and its durability. It should also be pointed out that antibiotics are recommended for the first 60 days after exposure providing additional protection during this period and that it is unclear how recall responses would affect protection.

By day 180 both antibody levels had continued to fall in all groups. The day 0, 14 arm had the lowest GMT while the group receiving 3 full doses had the highest level. After the booster dose at day 180 all but one subject developed higher levels of both antibodies than at any time during the primary series.

Several studies have reported high rates of seroconversion following immunization with AVA by the SC or IM routes[12–16]. In addition, an improved response to a schedule with longer dosing intervals has been previously reported[17, 18]. In the largest study addressing dosing schedules and reduced doses, the CDC sponsored trial of altered dosing of AVA compared SQ to IM dosing at 0, 2, and 4 weeks with a 6 month boost and to IM dosing at 0

and 4 weeks followed by the 6 month boost[3, 14]. Only a quantitative anti-PA IgG ELISA was used to evaluate the antibody response. Similar to what we report here, both the per cent with 4 fold rises in antibody and the GMT at 4 weeks were lower for the groups not receiving a dose at 2 weeks. At 8 weeks (the next time point evaluated), the study arm receiving only 2 doses (0, 4 weeks) had a lower antibody response than those receiving 3 doses IM or SQ. On this basis, the FDA recommended retaining current PEP protocol (3 doses administered SQ). In the follow-up publication of the trial reported by Marano, et. al. [14], the complete evaluation[16] again showed that the day 0, and 28 groups had a lower GMT than the 0, 14, 28 group.

The study reported here fills in some gaps from the CDC-sponsored study above[13, 16]. We were able to compare day 0, 14 to 0, 14, 28 schedules when all vaccines are administered by the SQ route. We were also able to evaluate antibody responses between weeks 4 and 8 that could be critical in an outbreak. By assessing immune responses at weekly intervals we learned that omitting the day 14 dose led to lower antibody responses through day 35; 54.2% had a 4 fold TNA NF₅₀ response in the 0, 28 day schedule compared to 100% immunized at days 0, 14, and 28. In contrast to the CDC-sponsored trial, however, we found that the day 0, 28 schedule elicited significantly higher antibody concentrations than the schedule of days 0, 14, 28 from day 42–56 for the anti-PA IgG ELISA and from day 42–180 for TNA NF₅₀. This difference in these findings may relate to the route of immunization.

The data reported here will be useful for establishing a vaccine schedule should an anthrax outbreak develop. Depending on the extent of the outbreak, rate of spread, effectiveness of antibiotics and availability of vaccine, the 0, 28 or 0, 14, 28 schedule may have advantages. The use of half doses or the 0, 14 schedule would not appear to provide the same level of protection as the other regimens tested.

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Figure 1.
 Progress of subjects through trial

Figure 2.

Antibody response over time:

A) Geometric mean titer of TNA NF₅₀ antibody

B) Geometric mean concentration of anti-PA IgG Enzyme Linked Immunosorbent Assay (ELISA)

Blood was obtained at specified time points for the 4 study arms evaluated and antibody titers determined as described in the methods.

Table 1

Demographics of per protocol study participants

	All Arms (N=328)	Study Arm A BioThrax (Full dose) Day 0,14 (N=82)	Study Arm B BioThrax (Full dose) Day 0, 28 (N=82)	Study Arm C BioThrax (Full dose) Day 0,14, 28 (N=82)	Study Arm D BioThrax (Half dose) Day0, 14, 28 (N=82)
Gender - N(%)					
Male	162 (49)	39 (48)	40 (49)	42(51)	41 (50)
Female	166(51)	43 (52)	42(51)	40 (49)	41 (50)
Ethnicity - N(%)					
Non-Hispanic	309 (94)	76 (93)	78 (95)	79 (96)	76 (93)
Hispanic	19(6)	6(7)	4(5)	3(4)	6(7)
Race - N(%)					
American Indian/Alaskan Native	0	0	0	0	0
Asian	9(3)	2(2)	3(4)	1(1)	3(4)
Hawaiian/Pacific Islander	0	0	0	0	0
Black/African American	55(17)	7(9)	14(17)	19(23)	15(18)
White/Caucasian	245 (75)	67 (82)	61 (74)	56 (68)	61 (74)
Multi-Racial	16(5)	6(7)	3(4)	5(6)	2(2)
Other/Unknown	3(1)	0	1(1)	1(1)	1(1)
Age (Years)					
Mean(STD)	35.7(11.2)	35.0(10.8)	36.7(11.7)	34.6(11.5)	36.5(11.1)
Median	33.1	32.7	33.0	31.7	35.7
Minimum, Maximum	(18.7,65.0)	(19.4,60.8)	(19.5,63.9)	(18.7,62.7)	(19.0,65.0)

Table 2

Selected Systemic and local site reactions by study group and vaccination

Study Group=Study Arm A: BioThrax (Full dose) Day 0,14														
Severity														
Primary Vaccination 1			Primary Vaccination 2			Any Primary Vaccination ^a .			Booster Vaccination.					
Reactogenicity	None N (%) ^b	Mild N (%)	Moderate N (%)	Severe N (%)	None N (%)	Mild N (%)	Moderate N (%)	Severe N (%)	None N (%)	Mild N (%)	Moderate N (%)	Severe N (%)	Mild N (%)	Severe N (%)
Systemic														
Muscle Aches	50 (75)	15(22)	2(3)	40 (62)	18(28)	7(11)	7(11)	22 (33)	36 (54)	22 (33)	9(13)	39 (65)	13(22)	8(13)
Any Systemic Symptoms	37 (55)	18(27)	12 (18)	28 (43)	27 (42)	10(15)	10(15)	22 (33)	26 (39)	19(28)		35 (58)	13(22)	12(20)
Local														
Pain at Injection Site	21 (31)	29 (43)	17(25)	20(31)	32 (49)	13 (20)	13 (20)	12 (18)	32 (48)	23 (34)		16 (27)	24 (40)	20 (33)
Tenderness at Injection Site	7(10)	38 (57)	22 (33)	7(11)	34 (52)	24 (37)	24 (37)	2(3)	35 (52)	30 (45)		11 (18)	33 (55)	16(27)
Redness (Measurement grade)	36 (54)	26 (39)	5(7)	32 (49)	19(29)	13 (20)	13 (20)	1 (2)	26 (39)	26 (39)	14(21)	1 (1)	55 (92)	4(7)
Swelling (Measurement grade)	47 (70)	16(24)	4(6)	37 (57)	17(26)	10(15)	10(15)	1 (2)	35 (52)	19 (28)	12(18)	1 (1)	52 (87)	3(5)
Any Local Symptoms	3(4)	33 (49)	31 (46)	1 (2)	28 (43)	35 (54)	35 (54)	1 (2)	1 (1)	22 (33)	43 (64)	1 (1)	5(8)	22 (37)
Study Group=Study Arm B: BioThrax (Full dose) Day 0, 28														
Severity														
Primary Vaccination 1			Primary Vaccination 2			Any Primary Vaccination ^a .			Booster Vaccination					
Reactogenicity	None N (%)	Mild N (%)	Moderate N (%)	Severe N (%)	None N (%)	Mild N (%)	Moderate N (%)	Severe N (%)	None N (%)	Mild N (%)	Moderate N (%)	Severe N (%)	Mild N (%)	Severe N (%)
Systemic														
Muscle Aches	49 (73)	16(24)	2(3)	47 (73)	14(22)	3(5)	3(5)	43 (64)	43 (64)	19 (28)	5(7)	44 (73)	14(23)	2(3)
Any Systemic Symptoms	38 (57)	20 (30)	9(13)	38 (59)	20 (31)	6(9)	6(9)	29 (43)	29 (43)	25 (37)	13(19)	36 (60)	21 (35)	3(5)
Local														
Pain at Injection Site	14(21)	41 (61)	12 (18)	16(25)	37 (58)	11 (17)	11 (17)	9(13)	38 (57)	20 (30)		21 (35)	29 (48)	9(15)
Tenderness at Injection Site	8(12)	44 (66)	15(22)	5(8)	44 (69)	15 (23)	15 (23)	4(6)	41 (61)	22 (33)		8(13)	42 (70)	9(15)
Redness (Measurement grade)	39 (58)	23 (34)	4(6)	1 (1)	33 (52)	14(22)	17(27)	31 (46)	18 (27)	17(25)		1 (1)	56 (93)	1 (2)
Swelling (Measurement grade)	48 (72)	17(25)	2(3)	38 (59)	20 (31)	5(8)	5(8)	1 (2)	34 (51)	27 (40)	5(7)	1 (1)	56 (93)	2(3)
Any Local Symptoms	4(6)	40 (60)	22 (33)	1 (1)	3(5)	28 (44)	32 (50)	1 (2)	2(3)	24 (36)	39 (58)	2(3)	36 (60)	16(27)

Study Group=Study Arm C: BioThrax (Full dose) Day 0, 14, 28														
Severity														
Primary Vaccination. 1					Primary Vaccination. 2					Primary Vaccination 3				
Any Primary Vaccination ^a .					Any Primary Vaccination					Booster Vaccination				
Reactogenicity	Mild		Severe		Mild	Severe		Mild	Severe		Mild	Severe		Mild
	None N (%)	Moderate N (%)	None N (%)	Moderate N (%)		None N (%)	Moderate N (%)		None N (%)	Moderate N (%)		None N (%)	Moderate N (%)	
Systemic	Muscle Aches	49 (72)	12 (18)	7(10)	46 (71)	13 (20)	6(9)	51 (84)	7 (11)	38 (56)	20 (29)	10(15)	36 (64)	14 6(11)
	Any Systemic Symptoms	33 (49)	24 (35)	11 (16)	32 (49)	23 (35)	10(15)	42 (69)	15 (25)	21 (31)	32 (47)	15(22)	31 (55)	16 8(14)
Local	Pain at Injection Site	14 (21)	42 (62)	12(18)	16 (25)	37 (57)	12 (18)	34 (56)	24 (39)	9(13)	39 (57)	20 (29)	19 (34)	27 10 (18)
	Tenderness at Injection Site	5(7)	45 (66)	18 (26)	6(9)	43 (66)	16(25)	14 (23)	43 (70)	1 (2)	2(3)	41 (60)	13 (23)	28 15(27)
	Redness (Measurement grade)	42 (62)	21 (31)	5(7)	39 (60)	12 (18)	11 (17)	39 (64)	15 (25)	31 (46)	19 (28)	14(21)	4(6)	50 5(9)
	Swelling (Measurement grade)	50 (74)	17 (25)	1 (1)	39 (60)	18 (28)	7(11)	46 (75)	13 (21)	37 (54)	21 (31)	9(13)	1 (1)	53 2(4)
	Any Local Symptoms	4(6)	40 (59)	24 (35)	4(6)	34 (52)	24 (37)	8(13)	41 (67)	2(3)	30 (44)	31 (46)	5(7)	8(14)
														29 18 (32)
														1 (2)
Study Group=Study Arm D: BioThrax (Half dose) Day 0, 14, 28														
Severity														
Primary Vaccination 1					Primary Vaccination 2					Primary Vaccination 3				
Any Primary Vaccination					Any Primary Vaccination					Booster Vaccination				
Reactogenicity	Mild		Severe		Mild	Severe		Mild	Severe		Mild	Severe		Mild
	None N (%)	Moderate N (%)	None N (%)	Moderate N (%)		None N (%)	Moderate N (%)		None N (%)	Moderate N (%)		None N (%)	Moderate N (%)	
Systemic	Muscle Aches	49 (73)	14 (21)	4(6)	48 (74)	14 (22)	3(5)	49 (80)	12 (20)	38 (57)	24 (36)	5(7)	37 (66)	15 4(7)
	Any Systemic Symptoms	35 (52)	23 (34)	9(13)	37 (57)	18 (28)	10(15)	35 (57)	25 (41)	22 (33)	29 (43)	15(22)	32 (57)	19 5(9)
Local	Pain at Injection Site	24 (36)	35 (52)	8(12)	26 (40)	35 (54)	4(6)	42 (69)	17 (28)	15 (22)	41 (61)	11 (16)	22 (39)	20 13 (23)
	Tenderness at Injection Site	15 (22)	42 (63)	10(15)	8(12)	43 (66)	14(22)	14 (23)	43 (70)	6(9)	41 (61)	20 (30)	11 (20)	30 14 (25)
	Redness (Measurement grade)	47 (70)	17 (25)	3(4)	41 (63)	15 (23)	9(14)	36 (59)	17 (28)	7(11)	21 (31)	12(18)	1 (1)	54 1 (2)
	Swelling (Measurement grade)	55 (82)	11 (16)	1 (1)	49 (75)	11 (17)	5(8)	41 (67)	15 (25)	5(8)	20 (30)	9(13)	52 (93)	2(4)

Effect of vaccine schedule on number and percent with 4 fold rise in TNA NF₅₀ antibody titer at different time points; Pairwise Difference in Proportions (Fisher's Exact Test)

Study Visit	Fisher P-Value	Arm A		Arm B		Arm C		Arm D	
		BioThrax (Full dose) Day 0, 14	N (%) ^a	BioThrax (Full dose) Day 0, 28	N (%)	BioThrax (Full dose) Day 0, 14, 28	N (%)	BioThrax (Half dose) Day 0, 14, 28	N (%)
Day 7	1.000	1/67(1.5)	0/59 (0.0)						
	1.000	1/67(1.5)			1/59(1.7)				
	1.000	1/67(1.5)						0/60 (0.0)	
	1.000		0/59 (0.0)	0/59 (0.0)	1/59(1.7)				
			0/59 (0.0)					0/60 (0.0)	
	0.496				1/59(1.7)			0/60 (0.0)	
Day 14	0.370	4/67 (6.0)	1/59(1.7)						
	0.370	4/67 (6.0)			1/59(1.7)				
	0.121	4/67 (6.0)						0/60 (0.0)	
	1.000		1/59(1.7)	1/59(1.7)	1/59(1.7)				
	0.496		1/59(1.7)					0/60 (0.0)	
	0.496				1/59(1.7)			0/60 (0.0)	
Day 21	0.032	26/67 (38.8)	12/59(20.3)						
	0.462	26/67 (38.8)			19/59(32.2)				
	0.032	26/67 (38.8)						12/60(20.0)	
	0.209		12/59(20.3)	12/59(20.3)	19/59(32.2)				
	1.000		12/59(20.3)					12/60(20.0)	
	0.148				19/59(32.2)			12/60(20.0)	
Day 28	0.000	63/67 (94.0)	13/59(22.0)						
	1.000	63/67 (94.0)			56/59 (94.9)				
	0.009	63/67 (94.0)						46/60 (76.7)	
	0.000		13/59(22.0)	13/59(22.0)	56/59 (94.9)				
	0.000		13/59(22.0)					46/60 (76.7)	

Study Visit Day	Fisher P-Value	Arm A	Arm B	Arm C	Arm D
		BioThrax (Full dose) Day 0, 14 N (%) ^a	BioThrax (Full dose) Day 0, 28 N (%)	BioThrax (Full dose) Day 0, 14, 28 N (%)	BioThrax (Half dose) Day 0, 14, 28 N (%)
	0.007			56/59 (94.9)	46/60 (76.7)
Day 35	0.000	65/67 (97.0)	32/59 (54.2)		
	0.498	65/67 (97.0)		59/59 (100)	
	0.083	65/67 (97.0)			53/60 (88.3)
	0.000		32/59 (54.2)	59/59 (100)	
	0.000		32/59 (54.2)		53/60 (88.3)
	0.013			59/59 (100)	53/60 (88.3)
Day 42	0.122	63/67 (94.0)	59/59(100)		
	0.122	63/67 (94.0)		59/59 (100)	
	0.683	63/67 (94.0)			58/60 (96.7)
			59/59(100)	59/59 (100)	
	0.496		59/59(100)		58/60 (96.7)
	0.496			59/59 (100)	58/60 (96.7)
Day 49	0.122	63/67 (94.0)	59/59(100)		
	0.122	63/67 (94.0)		59/59 (100)	
	1.000	63/67 (94.0)			56/60 (93.3)
			59/59(100)	59/59 (100)	
	0.119		59/59(100)		56/60 (93.3)
	0.119			59/59 (100)	56/60 (93.3)
Day 56	0.007	59/67(88.1)	59/59(100)		
	0.007	59/67(88.1)		59/59 (100)	
	0.568	59/67(88.1)			55/60 (91.7)
			59/59(100)	59/59 (100)	
	0.057		59/59(100)		55/60 (91.7)
	0.057			59/59 (100)	55/60 (91.7)
Day 63	0.002	57/67(85.1)	59/59(100)		
	0.002	57/67(85.1)		59/59 (100)	

Study Visit Day	Fisher P-Value	Arm A		Arm B		Arm C		Arm D	
		BioThrax (Full dose) Day 0, 14	N (%) ^a	BioThrax (Full dose) Day 0, 28	N (%)	BioThrax (Full dose) Day 0, 14, 28	N (%)	BioThrax (Half dose) Day 0, 14, 28	N (%)
	0.436		57/67(85.1)		59/59(100)		59/59 (100)		54/60 (90.0)
	0.027				59/59(100)				54/60 (90.0)
	0.027				59/59(100)				54/60 (90.0)
Day 70 [DBI]	0.001		56/67 (83.6)		59/59(100)				
	0.001		56/67 (83.6)				59/59 (100)		
	0.312		56/67 (83.6)						54/60 (90.0)
					59/59(100)		59/59 (100)		
	0.027				59/59(100)				54/60 (90.0)
	0.027						59/59 (100)		54/60 (90.0)
Day 84	0.000		44/67 (65.7)		58/59 (98.3)				
	0.000		44/67 (65.7)				58/59 (98.3)		
	0.014		44/67 (65.7)						51/60(85.0)
	1.000				58/59 (98.3)		58/59 (98.3)		
	0.017				58/59 (98.3)				51/60(85.0)
	0.017						58/59 (98.3)		51/60(85.0)
Day 100	0.000		34/67 (50.7)		54/59 (91.5)				
	0.000		34/67 (50.7)				56/59 (94.9)		
	0.011		34/67 (50.7)						44/60 (73.3)
	0.717				54/59 (91.5)		56/59 (94.9)		
	0.015				54/59 (91.5)				44/60 (73.3)
	0.002						56/59 (94.9)		44/60 (73.3)
Day 180	0.002		9/59(15.3)		23/54 (42.6)				
	0.000		9/59(15.3)				37/56(66.1)		
	0.000		9/59(15.3)						30/55 (54.5)
	0.021				23/54 (42.6)		37/56(66.1)		

Study Visit Day	Fisher P-Value	Arm A		Arm B		Arm C		Arm D	
		BioThrax (Full dose) Day 0, 14	N (%) ^a	BioThrax (Full dose) Day 0, 28	N (%)	BioThrax (Full dose) Day 0, 14, 28	N (%)	BioThrax (Half dose) Day 0, 14, 28	N (%)
	0.252		23/54 (42.6)						30/55 (54.5)
	0.247						37/56(66.1)		30/55 (54.5)
Day 201	1.000	59/59(100)	54/54(100)						
	1.000	59/59(100)			56/56 (100)				
	1.000	59/59(100)						55/55 (100)	
	1.000		54/54(100)	54/54(100)	56/56 (100)				
	1.000		54/54(100)					55/55 (100)	
	1.000				56/56 (100)			55/55 (100)	

The shaded areas highlight the pairwise comparisons that were significantly different (Fisher's Exact test)

^aN= number seroconverted and (%) = the percent seroconverted.

Effect of vaccine schedule on number and percent with 4 fold rise in Anti-Pa IgG Antibody Concentration at different time points: Pairwise Difference in Proportions (Fisher's Exact Test)

Study Visit	Fisher P-Value	Arm A BioThrax (Full dose) Day 0, 14 N (%) ^a	Arm B BioThrax (Full dose) Day 0, 28 N (%)	Arm C BioThrax (Full dose) Day 0, 14, 28 N (%)	Arm D BioThrax (Half dose) Day 0, 14, 28 N (%)
Day 21	0.008	25/67 (37.3)	9/59(15.3)		
	0.019	25/67 (37.3)		11/60(18.3)	
	0.019		9/59(15.3)	21/59(35.6)	
	0.040			21/59(35.6)	11/60(18.3)
Day 28	0.000	65/67 (97.0)	9/59(15.3)		
	0.001	65/67 (97.0)			46/60 (76.7)
	0.000		9/59(15.3)	57/59 (96.6)	
	0.000		9/59(15.3)		46/60 (76.7)
	0.002			57/59 (96.6)	46/60 (76.7)
Day 35	0.000	65/67 (97.0)	24/59 (40.7)		
	0.000		24/59 (40.7)	59/59 (100)	
	0.000		24/59 (40.7)		54/60 (90.0)
	0.027			59/59 (100)	54/60 (90.0)
Day 49	0.029	61/67(91.0)	59/59(100)		
	0.029	61/67(91.0)		59/59 (100)	
Day 56	0.014	60/67 (89.6)	59/59(100)		
	0.014	60/67 (89.6)		59/59 (100)	
Day 63	0.002	57/67(85.1)	59/59(100)		
	0.002	57/67(85.1)		59/59 (100)	
	0.013		59/59(100)		53/60 (88.3)
	0.013			59/59 (100)	53/60 (88.3)
Day 70	0.001	54/67 (80.6)	58/59 (98.3)		
	0.001	54/67 (80.6)		58/59 (98.3)	

Study Visit Day	Fisher P-Value	Arm A	Arm B	Arm C	Arm D
		BioThrax (Full dose) Day 0, 14 N (%) ^a	BioThrax (Full dose) Day 0, 28 N (%)	BioThrax (Full dose) Day 0, 14, 28 N (%)	BioThrax (Half dose) Day 0, 14, 28 N (%)
	0.061		58/59 (98.3)		53/60 (88.3)
	0.061			58/59 (98.3)	53/60 (88.3)
Day 84	0.000	44/67 (65.7)	56/59 (94.9)		
	0.000	44/67 (65.7)		57/59 (96.6)	
	0.047	44/67 (65.7)			49/60 (81.7)
	0.043		56/59 (94.9)		49/60 (81.7)
	0.016			57/59 (96.6)	49/60 (81.7)
Day 100	0.000	38/67 (56.7)	52/59(88.1)		
	0.000	38/67 (56.7)		54/59 (91.5)	
	0.007	38/67 (56.7)			48/60 (80.0)
	0.317		52/59(88.1)		48/60 (80.0)
	0.114			54/59 (91.5)	48/60 (80.0)
Day 180	0.005	4/59 (6.8)	15/54(27.8)		
	0.001	4/59 (6.8)		18/56(32.1)	
	0.003	4/59 (6.8)			16/55(29.1)
	0.680		15/54(27.8)	18/56(32.1)	

Only evaluations in which the pairwise comparison was significantly different (dark shade) or where there was a difference between the Anti-Pa IgG Antibody and the TNA NF50 antibody (light shaded area) are shown.

^aN= number seroconverted and (%) = the percent seroconverted.