# Efficacy of a human anthrax vaccine in guinea pigs, rabbits, and rhesus macaques against challenge by *Bacillus anthracis* isolates of diverse geographical origin

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## Abstract

The efficacy of a licensed human anthrax vaccine (Anthrax Vaccine Adsorbed (AVA)) was tested in guinea pigs, rabbits, and rhesus macaques against spore challenge by *Bacillus anthracis* isolates of diverse geographical origin. Initially, groups of Hartley guinea pigs were vaccinated at 0 and 4 weeks with AVA, then challenged intramuscularly at 10 weeks with spores from 33 isolates of *B. anthracis*. Survival among the vaccinated groups varied from 6 to 100%, although there were no differences in mean time to death among the groups. There was no correlation between isolate virulence and variable number tandem repeat category or protective antigen genotype identified. New Zealand white rabbits were then vaccinated with AVA at 0 and 4 weeks, and challenged at 10 weeks by aerosol with spores from six of the isolates that were highly virulent in vaccinated guinea pigs. AVA completely protected the rabbits from four of the isolates, and protected 90% of the animals from the other two isolates. Subsequently, two of these six isolates were then used to challenge rhesus macaques, previously vaccinated with AVA at 0 and 4 weeks and challenged at 10 weeks by aerosol. AVA protected 80 and 100% of the animals from these two isolates. These studies demonstrated that, although AVA confers variable protection against different *B. anthracis* isolates in guinea pigs, it is highly protective against these same isolates in both rabbits and rhesus macaques. Published by Elsevier Science Ltd.

*Keywords*: Anthrax; *Bacillus anthracis*; Vaccine; Rabbit; Guinea pig; Rhesus macaque

## 1. Introduction

The current U.S. human anthrax vaccine, Anthrax Vaccine Adsorbed (AVA), consists of aluminum hydroxide-adsorbed supernatant material, primarily protective antigen (PA), from fermentor cultures of a toxigenic, non-encapsulated isolate *of Bacillus anthracis*, V770-NP1-R [1,2]. In humans, vaccination with AVA calls for a series of six doses within 18 months, followed by yearly boosters. Although there are no human clinical data on the efficacy of AVA, a 4-year placebo-controlled study from the 1950s demonstrated that a vaccine similar to AVA afforded a significant degree of protection to humans [3,4].

Protection studies in different animal species yielded varied results. For example, AVA virtually fails to protect mice [5,6] or hamsters [7] against a parenteral challenge by virulent *B. anthracis* spores. Vaccine efficacy studies in guinea pigs showed that AVA only partially protected the animals from parenteral challenge from certain virulent isolates of *B. anthracis* [8-11]. Little and Knudson identified nine of 27 *B. anthracis* challenge isolates that appeared to overcome vaccination of guinea pigs with AVA [10]. Ivins et al. found that vaccination with AVA variably protected guinea pigs against challenge from two isolates of *B. anthracis*, Ames and Vollum 1B. The Ames isolate, however, was significantly more virulent in the vaccinated animals [11]. Recent vaccine studies demonstrated that rabbits and rhesus macaques are well protected by AVA from an aerosol challenge with the Ames isolate [12].

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These data reflect differences in disease pathogenesis, or intrinsic antibody response with respect to the animal model, and the immune response to AVA and anthrax pathogenicity as it relates to humans.

Although human cases of anthrax are relatively rare in the United States, other countries suffer from endemic outbreaks of the disease [13] and there is concern about the possible use of *B. anthracis* as a weapon [14]. Therefore, it is important to determine whether there are isolates of *B. anthracis* for which the vaccine is not efficacious. In order to clarify the relationship between AVA efficacy, animal models, and isolate diversity we systematically compared the efficacy of AVA in three animal models against challenge from a geographically diverse group of *B. anthracis* isolates. The guinea pig model was initially chosen to screen the isolates for several reasons. Historically, it has been the animal model most often used to test anthrax vaccine efficacy [15,16], and therefore it is the model for which there are the most data. Furthermore, guinea pigs have been demonstrated to be an appropriate animal model for use in determining differences in isolates of *B. anthracis* with respect to virulence in an immunized host [8-11]. The rabbit model was chosen for the second portion of these studies because of its similarity to rhesus macaques with respect to its ability to be highly protected by AVA [12] and the pathology that it demonstrates in experimental inhalational anthrax [17]. The third and final portion of these studies used rhesus macaques because the disease in these animals most closely resembles the infection in humans.

## 2. Materials and Methods

### 2.1. B. anthracis isolates

The isolates used in these studies are presented in Table 1.

### 2.2. Vaccine

AVA was supplied by Michigan Biological Product Institute, formally the Michigan Department of Public Health, currently BioPort Corporation (Lansing, MI). The lots of vaccine used in this study were FAV018 (guinea pigs), FAV032 (rabbits), and FAV038 (rhesus macaques).

### 2.3. Spore preparation

*B. anthracis* isolates were inoculated onto 5% sheep blood agar and incubated overnight at $37^{o}C$. The following day an inoculum was prepared by suspending a loopful of growth into 5 ml phosphate-buffered saline. Two-liter Erlenmeyer flasks containing 250 ml Leighton-Doi [18] broth were inoculated with 0.2 ml cell suspension and incubated at $37^{o}C$ for 3 days with moderate shaking. The spores were harvested by pelleting at 10000 x *g*. They were then washed twice in sterile water for injection (McGaw, Inc., Irvine, CA), suspended in 1% phenol and stored at $4^{o}C$. Spores prepared for intramuscular (i.m.) challenge were resuspended in sterile water for injection and then heat shocked at $60^{o}C$ for 45 min.

Table 1
*B. anthracis* isolates tested[a]

| Animal isolates | Human isolates | Other isolates |
|---|---|---|
| ASIL K0778/Canada | ASIL K4539/France | 33/South Africa[b] |
| ASIL K1963/Canada | BA1017/Haiti | ASIL K1769/South Africa[c] |
| ASIL K6286/Canada | ASIL K5926/India | BA1024/Ireland[d] |
| BA0018/Canada | ASIL K6387/India | |
| ASIL K6093/Croatia | BA1023/Pakistan | |
| ASIL K7282/Germany | ASIL K7038/South Korea | |
| ASIL K1938/Indonesia | 28 Ohio ASB/USA | |
| ASIL K4241/Italy | BA1086/Zimbabwe | |
| ASIL K4849/Mozambique | | |
| ASIL K7978/Namibia | | |
| ASIL K1671/Norway | | |
| ASIL K8091/Norway | | |
| BA1003/South Africa | | |
| BA1018/South Africa | | |
| BA1031/South Africa | | |
| ASIL K3519/Tanzania | | |
| ASIL K9729/Turkey | | |
| Ames/USA | | |
| ASIL K2087/USA | | |
| BA1007/USA | | |
| Texas-2/USA | | |
| BA1002/Vollum 1B | | |

[a]Isolates with the ASIL designation were kindly provided by the laboratory of Martin Hugh-Jones at Louisiana State University, Baton Rouge, LA. All other Isolates were obtained from the Bacteriology Division culture collection of the US Army Medical Research Institute of Infectious Diseases.
[b]Unknown origin.
[c]Environment isolate.
[d]Textile isolate.

The appropriate dilution for challenge was prepared in a 0.2 ml dose. Spores for aerosol challenge were purified further by centrifugation through 58% Hypaque-76 (Nycomed Inc, Princeton, NJ) [19], washed, resuspended in sterile water for injection and heat shocked at 60$^{\circ}$C for 45 min. The appropriate dilution for challenge was prepared in 8 ml aliquots for aerosol exposure.

### 2.4. Vaccination and challenge schedule

Hartley guinea pigs, New Zealand white rabbits and rhesus macaques were vaccinated at 0 and 4 weeks i.m. with 0.5 ml of AVA. All control animals received 0.5 ml of saline. Challenge occurred 10 weeks after the first vaccination and animals were monitored for survival for 14 days. Death by anthrax was confirmed in all animals by plating blood on tryptic soy agar (TSA) and incubating overnight at 37$^{\circ}$C.

### 2.5. Parenteral challenge in guinea pigs

Hartley guinea pigs (Charles River, Wilimington, MA) previously vaccinated with AVA (300-350 g) in groups of eight males and eight females were challenged i.m. with approximately 10000 B. anthracis spores (100 LD$_{50}$ Ames equivalents) and survival was noted for 14 days. Although their virulence has been extensively studied in vaccinated guinea pigs [8-11,15,16,19-21], the Vollum 1B and Ames isolates were also tested in the initial screening. Seven isolates that killed all but one or two of the vaccinated animals were then tested in a subsequent experiment in which the i.m. challenge dose was decreased to 5000 spores (50 Ames LD$_{50}$ equivalents).

### 2.6. Aerosol challenge in rabbits and non-human primates

Six of the isolates found to be most virulent in vaccinated guinea pigs were selected for testing in New Zealand white rabbits (Charles River, Wilimington, MA), (2.5-3.5 kg) five males and five females per group. Subsequently, two isolates were selected for testing in rhesus macaques

(USAMRIID non-human primate colony) (4.0-6.0 kg), five males and five females per group. Minute respiratory volumes were measured on both rabbits and monkeys just before challenge. The rabbits were exposed in a nose-only chamber and non-human primates in a head-only chamber to a spore aerosol generated by a three-jet Collison nebulizer [22-24]. The concentration of spores in the aerosol inhaled dose (expressed as LD$_{50}$) was calculated on all animals after plating diluted samples from the all glass impinger onto TSA plates (Difco, Detroit, MI). Non-human primates were observed at least twice daily with regard to their appetite, activity, behavior, response to stimuli, and respiratory distress.

### 2.7. Serology studies

Rabbits were bled at 9 weeks and rhesus macaques bled at 6 weeks, and sera tested for antibodies to PA by direct ELISA as described by Iacono-Connors et al. [25]. The 6-week enzyme-linked immunosorbent assay (ELISA) titers represent the peak antibody response in rhesus macaques vaccinated at 0 and 4 weeks with AVA (L. Pitt, unpublished data). Briefly, purified recombinant PA was adsorbed directly onto the surface of polyvinyl chloride microtiter wells. After the addition of serially diluted serum samples, bound immunoglobulin (IgG) was measured with horseradish peroxidase-conjugated antibody directed against species specific IgG.

The mean adjusted OD values were determined for negative control samples and the standard deviation was calculated. The cut-off of the assay was the mean OD value plus three standard deviations rounded up to the nearest tenth. Extinction titers were determined by drawing a best fit line through all points (OD values) ranging from an OD value of 20 through one point past the last OD value which was equal to or greater than the cut-off value. Using this defined line, the reciprocal of the dilution of the sera that corresponds to the cut-off value was called the extinction titer. Microsoft Excel 5.0 software and forecast formula were used for these analyses. Geometric mean anti-PA endpoint ELISA titers for each group were determined. The coefficient of variation of this assay was <20%. Guinea pigs were not bled for determination of ELISA anti-PA titers.

## 2.8. Bacteremia studies

After challenge, approximately 1 ml of blood was drawn on day 2 postchallenge in the rabbits and daily for 1 week after challenge in the rhesus macaques. Aliquots (0.1 ml) of serial dilutions of the blood were plated in triplicate onto TSA. The plates were incubated for 18 h at 37°C and *B. anthracis* colonies counted.

## 2.9. Statistical analysis

The arithmetic mean time to death (TTD) in the vaccinated and control guinea pigs was determined for each challenge isolate. Survival data were analyzed by using the log-rank test to compare time of death and survival between genders. The Fisher exact test was used to compare final survival totals in the guinea pig. Arithmetic mean TTD was also determined in both control rabbit and rhesus macaques for each challenge isolate.

## 3. Results

The Ames isolate of *B. anthracis* was previously described as 'vaccine- resistant' in vaccinated guinea pigs [10,11], and this study corroborates this finding, with only two of 16 (13%) of vaccinated animals surviving a 10000 spore challenge (100 $LD_{50}$ Ames equivalent). Eight additional *B. anthracis* isolates were identified that were as virulent as the Ames isolate in guinea pigs vaccinated with AVA (Table 2). All but one vaccinated animal challenged with ASIL K4539/France, BA1086/Zimbabwe, and ASIL K7978/Namibia was killed. Of the control guinea pigs challenged with these highly virulent isolates (two males and two females per challenge isolate), all but one died (data not shown). There were no statistically significant differences in the mean times to death among the groups of vaccinated guinea pigs that died.

More female than male guinea pigs survived when challenged with the various isolates of *B. anthracis*. When all groups were combined, there was a significant difference ($P < 0.0001$) in survival between female and male guinea pigs, with 44% of females and 23% of males surviving challenge. However, there was no significant difference ($P = 0.2633$) between genders with respect to mean times to death. Thus, although more males than females died, they did not die more rapidly.

In a subsequent experiment, vaccinated guinea pigs were challenged with seven of the eight most highly virulent isolates using a lower dose of 5000 spores. Fewer than 20% of guinea pigs challenged with BA1086/Zimbabwe, ASIL K9729/Turkey and ASIL K8091/Norway survived challenge (Table 3). ASIL K5926/India was the only isolate tested at this dose that killed fewer than one-half of the vaccinated guinea pigs challenged with 5000 spores.

Six isolates exhibiting the greatest virulence in vaccinated guinea pigs were then tested in AVA-vaccinated rabbits. BA1086/Zimbabwe was not tested in these rabbit aerosol challenge studies because of the rapidity with which its spores declined in refractility and viability. Male and female rabbits were highly protected from aerosol challenge with spores from all six *B. anthracis* isolates (Table 4). All control rabbits (five males and five females per group) died, with TTD ranging from 1.7 to 2.5 days, and an overall mean TTD of 2.1 days. Vaccinated rabbits were completely protected from challenge with isolates ASIL K5926/India, ASIL K4539/France, ASIL K9729/Turkey and ASIL K1938/Indonesia. Two isolates, ASIL K7978/Namibia and ASIL K8091/Norway, killed one rabbit each. Although, low-level bacteremia (<100 CFU/ml) resulted after challenge with the majority of isolates, ASIL K9729/Turkey and ASIL K7978/Namibia exhibited a high incidence of bacteremia. ELISA data (Table 5) indicated that vaccination with AVA elicited a strong anti-PA response in both males and females. Spore challenge resulted in a boost in PA titers in challenge groups. Although the rise in PA ELISA titers after challenge was greater in females than in males, this difference was not significant. Likewise, there was no statistical difference in titer before challenge between male and female rabbits.

The two isolates that produced the highest incidence of bacteremia in rabbits, Namibia and Turkey, were further tested in rhesus macaques. AVA provided 100% protection against aerosol challenge with the Namibia isolate and 80% protection against aerosol challenge with the Turkey isolate (Table 6). All control animals (five males and five females) in both groups died between 2 and 4 days post-challenge. None of the vaccinated animals that survived challenge exhibited any obvious signs of infection. Although all vaccinated rhesus macaques survived challenge with the Namibia isolate, three demonstrated transient low-level bacteremia

(<100 CFU/ml). Bacteremia was seen in three rhesus macaques challenged with the Turkey isolate. One animal had a low-level bacteremia (<100 CFU/ml) on day 6 and survived. Of the two animals that died from challenge with the Turkey isolate, one had no detectable level of bacteremia until the time of death on day 9 postchallenge. The other animal had $10^3$ CFU/ml on day 2 post-challenge, <100 CFU/ml on day 3, and died on day 4 post-challenge with a terminal bacteremia of $10^5$ CFU/ml. ELISA data (Table 7) indicated that vaccination with AVA elicited a strong anti-PA response from both males and females. There was no statistical difference ($P = 0.0568$) in prechallenge titers between the males and females. However, spore challenge boosted the titers of both groups which was significant ($P < 0.05$) in the female rhesus macaques, but not in the males.

## 4. Discussion

Male and female guinea pigs that were vaccinated with two doses of AVA and then challenged with virulent isolates of *B. anthracis* from diverse host species and geographical origins demonstrated varying degrees of survival. Eight of thirty-three isolates were identified which exhibited a level of virulence equal to or greater than that of the *B. anthracis* Ames isolate, which has been previously described as 'vaccine-resistant' in vaccinated guinea pigs [10,11]. The data in this study are in agreement with this finding. The basis of this high level of virulence in vaccinated guinea pigs is not well understood. However, Welkos and Friedlander [6] showed in a mouse model that differences in virulence among isolates of *B. anthracis* are both plasmid and chromosome-mediated. Sex-related differences in resistance to infectious diseases have been reported with females generally being more resistant than males [26,27] This may also occur in anthrax infections. Elkin et al. [28] recently reported after an analysis of mortality records from nine documented naturally occurring anthrax outbreaks in bison between 1962 and 1993, a substantially higher proportion of deaths among males. Although no definitive reason for this difference was determined, it may be that the innate immune response was greater in females than in males. In unvaccinated guinea pigs, rabbits, and

rhesus macaques, we failed to observe gender based differences in survival after anthrax spore challenge. However, in our laboratory, we observed that female guinea pigs vaccinated with AVA develop a stronger antibody response to PA as determined by ELISA (P. Fellows, unpublished data). We observed no gender difference in the antibody response of rabbits and

Table 2
Efficacy of AVA against 10 000 spore i.m. challenge in guinea pigs[a]

| Isolate | Survivors/total | % Survival | TTD[b] |
|---|---|---|---|
| *Human isolates* | | | |
| ASIL K4539/France | 1/16 | 6 | 4.67 |
| BA1086/Zimbabwe | 1/15 | 7 | 4.4 |
| ASIL K5926/India | 2/16 | 13 | 5.0 |
| ASIL K7038/South Korea | 3/16 | 19 | 4.4 |
| ASIL K6387/India | 4/16 | 25 | 5.25 |
| 28 Ohio ASB/USA | 5/16 | 31 | 6.0 |
| BA1017/Haiti | 5/16 | 31 | 5.8 |
| BA1023/Pakistan | 8/16 | 50 | 5.25 |
| | | | |
| *Animal isolates* | | | |
| ASIL/K7978/Namibia | 1/16 | 6 | 4.6 |
| Ames/USA | 2/16 | 13 | 4.0 |
| ASIL K8091/Norway | 2/16 | 13 | 5.2 |
| ASIL K9729/Turkey | 2/16 | 13) | 4.43 |
| ASIL K1938/Indonesia | 2/16 | 13 | 4.29 |
| Texas-2/USA | 3/16 | 19 | 5.8 |
| BA1031/South Africa | 4/16 | 25 | 4.67 |
| BA1018/South Africa | 5/16 | 31) | 5.6 |
| ASIL K3519/Tanzania | 5.16 | 31 | 3.6 |
| ASIL K4241/Italy | 6/16 | 38 | 6.0 |
| ASIL K1963/Canada | 6/16 | 38 | 7.0 |
| ASIL K1671/Norway | 7/16 | 44 | 6.8 |
| ASIL K4849/Mozambique | 7/16 | 44 | 5.67 |
| ASIL K6093/Croatia | 7/16 | 44) | 5.2 |
| ASIL K0778/Canada | 8/16 | 50 | 4.63 |
| ASIL K2087/USA | 8/16 | 50 | 5.5 |
| ASIL K7282/Germany | 9/16 | 56 | 4.57 |
| BA1002/Vollum 1B | 9/16 | 56 | 6.7 |
| BA1007/USA | 9/16 | 56 | 6.3 |
| ASIL K6286/Canada | 12/16 | 75 | 4.5 |
| BA0018/Canada | 16/16 | 100 | NA[c] |
| | | | |
| *Other isolates* | | | |
| 33/South Africa | 2/16 | 13 | 6.5 |
| ASIL K1769/South Africa | 4/16 | 25 | 4.5 |
| BA1024/Ireland | 7/16 | 44 | 7.33 |
| BA1033/South Africa | 16/16 | 100 | NA[c] |

[a] Animals were vaccinated at 0 and 4 weeks, and were i.m. challenged at 10 weeks with 100 Ames equivalent $LD_{50s}$ (parenteral Ames $LD_{50}$ in Hartley Guinea pigs equals approximately 100 spores).
[b] TTD, Arithmetic mean time to death.
[c] NA, non-applicable.

Table 3
Efficacy of AVA against 5000 spore i.m. challenge in guinea pigs[a]

| Isolate | Survivors/total | % Survival | TTD (days)[b] |
|---|---|---|---|
| BA1086/Zimbabwe | 2/16 | 13 | 6.0 |
| ASIL K9729/Turkey | 2/16 | 13 | 5.6 |
| ASIL K8091/Norway | 3/16 | 19 | 4.9 |
| ASIL K4539/France | 4/16 | 25 | 5.6 |
| ASIL K1938/Indonesia | 7/16 | 44 | 6.2 |
| ASIL K7978/Namibia | 7/15 | 47 | 4.4 |
| ASIL K5926/India | 9/16 | 56 | 5.8 |

[a]Animals were vaccinated at 0 and 4 weeks, and i.m. challenged at 10 weeks.
[b]TTD, Arithmmetic mean time to death.

Table 5
ELISA titers in rabbits[a]

| | Females | Males | Combined |
|---|---|---|---|
| Prechallenge[b] | 1 591 511 | 1 579 091 | 1 585 289 |
| Postchallenge[c] | 3 785 110 | 1 918 001 | 2 694 410 |

[a] Reciprocal geometric mean titers to PA.
[b] Titers 1 week before challenge.
[c] Titers 3 weeks after challenge.

rhesus macaques to AVA vaccinations (Tables 5 and 7).

In contrast to guinea pigs, rabbits were protected by vaccination with AVA. This is significant in that the immunized rabbits survived aerosol challenge doses of spores that were equivalent to 300-2700 aerosol $LD_{50s}$ of the Ames isolate. Although the animals were protected from death at these high challenge doses, several demonstrated a transient bacteremia. This is noteworthy in that previous studies in which rabbits were vaccinated with AVA and challenged with the Ames isolate, no bacteremia was found in any of the rabbits [29]. However, because $LD_{50}$ determinations were not performed for the isolates in these studies, it is unknown whether the reported bacteremias were the result of high challenge doses or of other unidentified factors.

Rhesus macaques were also well protected against an aerosol challenge. Indeed, no anthrax vaccine resistant isolates have been demonstrated in the non-human primate. The animals in this study received challenge doses of 400 and 1000 Ames $LD_{50}$ equivalents, respectively, for *B. anthracis* Namibia and Turkey isolates. Although these two isolates also elicited bacteremia in some of the rhesus macaques, the number of animals becoming bacteremic was less than those in the rabbits. These results confirm previous reports showing that AVA is highly protective in this non-human primate animal model [30-32].

The data presented in this study highlight the difference in AVA vaccine efficacy in different animal models. Although AVA-induced protection was variable in guinea pigs against different isolates, there was broad, high-level protection in both rabbits and rhesus macaques against the all isolates tested. The reasons for these host response differences are unknown, but could relate to differences in pathogenesis or, more likely, an impaired response of guinea pigs to AVA. This is supported by the fact that guinea pigs can be fully protected against challenge with the Ames isolate when PA is combined

Table 4
Efficacy of AVA in rabbits aerosol challenged with *B. anthracis* spores[a]

| Isolate | Mean inhaled dose | Ames $LD_{50}$ equivalents[b] | Vaccinated rabbits | | Controls |
|---|---|---|---|---|---|
| | | | Number of bacteremmic/total | Survivors/total | Survivors/total |
| ASIL K7978/Namibia | $1.37 \times 10^8$ | 1305 | 8/10 | 9/10 | 10/10 |
| ASIL K5926/India | $1.52 \times 10^8$ | 1448 | 1/10 | 9/9 | 10/10 |
| ASIL K8091/Norway | $3.78 \times 10^7$ | 360 | 0/10 | 9/10 | 10/10 |
| ASIL K4539/France | $1.25 \times 10^8$ | 1191 | 1/10 | 10/10 | 10/10 |
| ASIL K9729/Turkey | $8.3 \times 10^7$ | 790 | 7/10 | 10/10 | 10/10 |
| ASIL K1938/Indonesia | $2.88 \times 10^8$ | 2743 | 1/10 | 10/10 | 10/10 |

[a] New Zealand White rabbits were vaccinated at 0 and 4 weeks, then challenged by aerosol with spores of the six indicated isolates. Survival/death was noted for 14 days.
[b] Calculated Ames $LD_{50}$ equivalents of the mean inhaled dose. Ames aerosol $LD_{50}$ in New Zealand White rabbits equals approximately $10^5$ spores.

Table 6
Efficacy of AVA in rhesus macaques aerosol challenged with *B. anthracis* spores[a]

| Isolate | Mean inhaled dose | Ames LD50 equivalents[b] | Vaccinated | | Controls |
|---|---|---|---|---|---|
| | | | Number of bacteremic/total | Survivors/total | Survivors/total |
| ASIL K7978/Namibia | $2.19 \times 10^7$ | 398 | 3/10 | 10/10 | 2/2 |
| ASIL K9729/Turkey | $5.52 \times 10^7$ | 1004 | 2/10 | 8/10 | 2/2 |

[a] Rhesus macaques were vaccinated at 0 and 4 weeks, then challenged by aerosol with spores of either *B. anthracis* isolate Namibia or Turkey.
[b] Calculated Ames $LD_{50}$ equivalents of the mean inhaled dose. Ames aerosol $LD_{50}$ in non-human primates equals approximately $5.5 \times 10^4$ spores.

with other adjuvants, rather that the aluminum hydroxide adjuvant used in AVA [21]. Thus, these results emphasize the importance of examining multiple animal species in an attempt to model the effectiveness of vaccines for humans.

## Acknowledgements

Table 7
ELISA titers in rhesus macaques[a]

| | Females | Males | Combined |
|---|---|---|---|
| Prechallenge[b] | 8445 | 16 889 | 11 943 |
| Postchallenge[c] | 25 600 | 23 702 | 24 633 |

[a] Reciprocal geometric mean titers to PA.
[b] Titers at 6 weeks.
[c] Titers 4 weeks after challenge.

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