Cochrane
Library

Cochrane Database of Systematic Reviews

Vaccines for preventing anthrax (Review)

Donegan S, Bellamy R, Gamble CL

Donegan S, Bellamy R, Gamble CL.
Vaccines for preventing anthrax.
Cochrane Database of Systematic Reviews 2009, Issue 2. Art. No.: CD006403.
DOI: [10.1002/14651858.CD006403.pub2](https://doi.org/10.1002/14651858.CD006403.pub2).

www.cochranelibrary.com

TABLE OF CONTENTS

HEADER	1
ABSTRACT	1
PLAIN LANGUAGE SUMMARY	2
BACKGROUND	3
OBJECTIVES	4
METHODS	4
RESULTS	5
Figure 1.	8
DISCUSSION	10
AUTHORS' CONCLUSIONS	11
ACKNOWLEDGEMENTS	11
REFERENCES	12
CHARACTERISTICS OF STUDIES	13
APPENDICES	26
WHAT'S NEW	38
HISTORY	38
CONTRIBUTIONS OF AUTHORS	38
DECLARATIONS OF INTEREST	38
SOURCES OF SUPPORT	38
DIFFERENCES BETWEEN PROTOCOL AND REVIEW	39
INDEX TERMS	39

[Intervention Review]

Vaccines for preventing anthrax

Sarah Donegan¹, Richard Bellamy², Carrol L Gamble³

¹International Health Group, Liverpool School of Tropical Medicine, Liverpool, UK. ²Department of Infection and Travel Medicine, James Cook University Hospital, Middlesbrough, UK. ³Centre for Medical Statistics and Health Evaluation, University of Liverpool, Liverpool, UK

Contact address: Sarah Donegan, International Health Group, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, Merseyside, L3 5QA, UK. Sarah.Donegan@liverpool.ac.uk.

Editorial group: Cochrane Infectious Diseases Group.

Publication status and date: Unchanged, published in Issue 5, 2019.

Citation: Donegan S, Bellamy R, Gamble CL. Vaccines for preventing anthrax. *Cochrane Database of Systematic Reviews* 2009, Issue 2. Art. No.: CD006403. DOI: [10.1002/14651858.CD006403.pub2](https://doi.org/10.1002/14651858.CD006403.pub2).

Copyright © 2019 The Cochrane Collaboration. Published by John Wiley & Sons, Ltd.

ABSTRACT

Background

Anthrax is a bacterial zoonosis that occasionally causes human disease and is potentially fatal. Anthrax vaccines include a live-attenuated vaccine, an alum-precipitated cell-free filtrate vaccine, and a recombinant protein vaccine.

Objectives

To evaluate the effectiveness, immunogenicity, and safety of vaccines for preventing anthrax.

Search methods

We searched the following databases (November 2008): Cochrane Infectious Diseases Group Specialized Register; CENTRAL (*The Cochrane Library* 2008, Issue 4); MEDLINE; EMBASE; LILACS; and mRCT. We also searched reference lists.

Selection criteria

We included randomized controlled trials (RCTs) of individuals and cluster-RCTs comparing anthrax vaccine with placebo, other (non-anthrax) vaccines, or no intervention; or comparing administration routes or treatment regimens of anthrax vaccine.

Data collection and analysis

Two authors independently considered trial eligibility, assessed risk of bias, and extracted data. We presented cases of anthrax and seroconversion rates using risk ratios (RR) and 95% confidence intervals (CI). We summarized immunoglobulin G (IgG) concentrations using geometric means. We carried out a sensitivity analysis to investigate the effect of clustering on the results from one cluster-RCT. No meta-analysis was undertaken.

Main results

One cluster-RCT (with 157,259 participants) and four RCTs of individuals (1917 participants) met the inclusion criteria. The cluster-RCT from the former USSR showed that, compared with no vaccine, a live-attenuated vaccine (called STI) protected against clinical anthrax whether given by a needleless device (RR 0.16; 102,737 participants, 154 clusters) or the scarification method (RR 0.25; 104,496 participants, 151 clusters). Confidence intervals were statistically significant in unadjusted calculations, but when a small amount of association within clusters was assumed, the differences were not statistically significant. The four RCTs (of individuals) of inactivated vaccines (anthrax vaccine absorbed and recombinant protective antigen) showed a dose response relationship for the anti-protective antigen IgG antibody titre. Intramuscular administration was associated with fewer injection site reactions than subcutaneous injection, and injection site reaction rates were lower when the dosage interval was longer.

Authors' conclusions

One cluster-RCT provides limited evidence that a live-attenuated vaccine is effective in preventing cutaneous anthrax. Vaccines based on anthrax antigens are immunogenic in most vaccinees with few adverse events or reactions. Ongoing randomized controlled trials are investigating the immunogenicity and safety of anthrax vaccines.

8 May 2019

No update planned

Other

The vaccine is the only one available and is currently recommended by the CDC and others for people at high risk of exposure, such as health officials inspecting animals and staff who slaughter animals. It is not a priority for the CIDG for review update.

PLAIN LANGUAGE SUMMARY

Vaccines for preventing anthrax

Anthrax is a bacterial infection that usually affects animals. Anthrax is not common in humans, but it can be acquired through breaks in the skin, from contaminated food, and through inhalation of bacteria. Human anthrax is often serious and can cause high rates of death. Various types of anthrax vaccines have been developed to protect those who may be exposed to the infection. The authors of this review wanted to evaluate the benefits and harms of vaccines for preventing anthrax. They identified four recent smaller randomized controlled trials of individuals and an older large cluster-randomized controlled trial with over 150,000 participants. The cluster trial provided limited evidence that a vaccine, based on a strain of live anthrax organisms incapable of causing disease, was effective in preventing cutaneous anthrax. More recent types of vaccines tested in the smaller trials, also based on inactivated components of the anthrax bacterium, appear to have few adverse events and to stimulate a good immune response. Several randomized controlled trials testing these newer vaccines are currently in progress. They will provide further information on the immunogenicity and safety of different vaccine regimens to be used for people at risk of anthrax exposure.

BACKGROUND

Anthrax is a bacterial disease that primarily affects herbivores. It is caused by *Bacillus anthracis*, an aerobic Gram-positive bacterium that produces hardy spores. *B. anthracis* spores infect herbivores by inhalation or ingestion. Infected animals shed anthrax bacilli from body fluids, and these bacilli produce spores when exposed to the external environment. These spores can survive for many years in certain types of soil. They can be found in wool, hair, animal hides, bone, and the carcasses of infected animals. The spores are predominantly responsible for the spread of the infection to herbivores and other animals, including humans.

In humans, anthrax can cause skin infections or, less commonly, infections of the gastrointestinal or respiratory tracts.

Cutaneous infection occurs when anthrax spores enter skin abrasions. Respiratory infection is caused by inhalation of spores (eg from contaminated goat hair). Gastrointestinal infection is caused by ingestion of spores or possibly of vegetative bacteria in contaminated foodstuffs. The cutaneous form accounts for about 95% of human infections. It starts as a skin ulcer that heals after one to two weeks leaving a coal-black plug (eschar), usually one to three centimetres in diameter. About 10% of cases of cutaneous anthrax progress to systemic disease unless treated with antibiotics (Dixon 1999; Inglesby 2002).

The respiratory form initially presents with non-specific symptoms of malaise, fever, cough, breathlessness, headache, vomiting, and abdominal and chest pain. These symptoms last between a few hours and several days. The patient may then rapidly progress to the septicæmic phase of the illness or there may be a brief period of recovery first. The fulminant second stage of the illness develops rapidly causing severe breathlessness, fever, and shock. Inhalational anthrax causes massive enlargement of the lymph nodes in the mediastinum (contains the heart, area surrounding the heart, and great vessels of the heart). Massive bleeding into the mediastinum is typically present in fatal cases. Clinical deterioration is rapid and death may occur within a few hours.

Ingestion of anthrax bacteria can cause two forms of disease. In the oropharyngeal form, there are oral and oesophageal ulcers, mucosal oedema, and regional lymphadenopathy that lead to septicæmia. The lower gastrointestinal form presents with nausea, vomiting, bloody diarrhoea, acute abdominal pain, and septicæmia.

Death in all forms of the disease is usually from septicæmia. In up to one half of cases there is also haemorrhagic meningitis, which is often only identified post-mortem (Inglesby 2002). The inhalational and gastrointestinal forms of the disease have high case-fatality rates, with estimates of untreated cases resulting in death of 100% (Dixon 1999) and 25% to 60% (Inglesby 2002) respectively. The cutaneous form of the disease has been reported to be fatal in up to 20% of untreated cases (Inglesby 2002).

Anthrax is rare in humans, with an estimated 20,000 to 100,000 cases occurring each year worldwide. Most cases are concentrated in agricultural regions where people raise livestock, such as in South and Central America, Southern and Eastern Europe, the Caribbean, and the Middle East (Dixon 1999). There is believed to be no risk of person-to-person spread for inhalational or gastrointestinal anthrax. Anthrax can very rarely spread from

person to person in the cutaneous form of the disease. The diagnosis of anthrax is confirmed by isolation of *B. anthracis* from the blood, cerebrospinal fluid, pleural fluid, cutaneous vesicle fluid, or other specimens. If the laboratory is not alerted to the possibility of anthrax, *B. anthracis* may not be correctly identified.

Interventions against anthrax include prevention through education of operators, dust control, environmental decontamination, and vaccination, and treatment with antibiotics. Human vaccines against the bacillus were developed in the 1950s and 1960s, and are thought to work through their capacity to stimulate immunity to three antigens: protective antigen (PA); lethal factor (LF); and oedema factor (EF). These three antigens are responsible for the pathogenicity of the anthrax bacillus.

The anthrax vaccines that have been developed include the Georgian/Russian vaccine, the UK vaccine, and the USA vaccine. Antibodies against PA are thought to confer protection, making PA a good candidate for a vaccine. A recombinant PA vaccine has now been developed (rPA102; VaxGen Inc., Brisbane, California), and several clinical studies have commenced. The Georgian/Russian vaccine (Tbilisi Research Institute of Vaccines and Serums, Republic of Georgia) consists of live spores from a Stern strain of *B. anthracis*. This vaccine is administered in the shoulder by scarification. The UK and USA vaccines consist of an alum-precipitated, cell-free filtrate of bacilli (sometimes called supernatant particles). The USA vaccine (BioThrax: Emergent BioSolutions) is adsorbed onto aluminium-hydroxide (so called adsorbed anthrax vaccine, or AVA). The vaccine is administered by three subcutaneous injections of 0.5 mL with intervals of two weeks between them, followed by an additional three injections at six, 12, and 18 months after the initial dose. The UK vaccine is given as an intramuscular injection by three 0.5 mL doses with a three-week interval between each injection. A fourth dose is given at least six months after the initial immunization course. A booster dose is recommended annually for AVA vaccines (Salisbury 1996). In the UK, inactivated anthrax vaccine is recommended only for workers at risk of exposure to anthrax, such as personnel of tanneries processing wool or hides and bone. Vaccination of UK and USA military personnel has also been undertaken due to concern anthrax may be used as a biological weapon.

Local adverse effects of anthrax vaccination include slight tenderness and redness of the upper arm (injection site), which uncommonly may be severe and extend to include the forearm. Systemic reactions occur in less than 0.2% of recipients (CDC 2006a).

Based on case reports and epidemiological studies, a report by the US Institute of Medicine Committee to Assess the Safety and Efficacy of the Anthrax Vaccine stated that anthrax vaccine is safe and effective, but needs improvement (Jollenbeck 2002). We have prepared a systematic review of the potential benefits and harms of the available anthrax vaccines because further studies have been reported or commenced since that time. The present-day low prevalence of anthrax infection among humans precludes controlled clinical studies that evaluate anthrax disease as an outcome. Trials in recent years have used antibody responses to the PA antigen as a surrogate marker for protection because they have been shown to correlate with primary protection in rabbits and guinea pigs (Pitt 2001; Reuveny 2001). Seroconversion rate has frequently been used to estimate the proportion of vaccine recipients that produce an antibody response. Mean (or

median) serum anti-PA immunoglobulin G (IgG) antibody titres have been measured to quantify the degree of immunogenicity. This is particularly useful when comparing the response to two different vaccines or for one vaccine administered by different routes or dosage schedules.

OBJECTIVES

To evaluate the effectiveness, immunogenicity, and safety of vaccines for preventing anthrax.

METHODS

Criteria for considering studies for this review

Types of studies

Randomized controlled trials of individuals and cluster-randomized controlled trials.

Types of participants

Adults or children irrespective of immune status or special risk category.

Types of interventions

Intervention

Live-attenuated (capable of reproduction but not of causing the disease) or killed (neither capable of reproduction nor of causing the disease) anthrax vaccine administered by any route.

Control

Placebo, other non-anthrax vaccine, or no intervention; or anthrax vaccine given in another dose, another treatment regimen, or by another route.

Types of outcome measures

Primary

Cases of anthrax, including cutaneous, inhalation, and gastrointestinal forms of the disease. A case is defined by clinical illness confirmed by identification of *B. anthracis* in smear or culture.

Secondary

Seroconversion rates and increased specific antibody titres against anthrax antigens.

Adverse events

- Serious adverse events, such as life-threatening events that may lead to death, events requiring inpatient hospitalization or prolongation of existing hospitalizations, or events resulting in significant disability.
- Other adverse events, for instance, events resulting in discontinuation of the vaccine course.

Search methods for identification of studies

We attempted to identify all relevant trials regardless of language or publication status (published, unpublished, in press, and ongoing).

We searched the following databases using the search terms and strategy described in [Appendix 1](#): Cochrane Infectious Diseases

Group Specialized Register (November 2008); Cochrane Central Register of Controlled Trials (CENTRAL), published in *The Cochrane Library* (2008, Issue 4); MEDLINE (1966 to November 2008); EMBASE (1974 to November 2008); and LILACS (1982 to November 2008).

The *metaRegister* of Controlled Trials (*mRCT*) was searched using [anthrax] as the search term (November 2008).

We checked the reference lists of all studies identified by the above methods.

Data collection and analysis

Selection of studies

SD and CG scanned the results of the literature search for potentially relevant trials and retrieved the corresponding full articles. SD and CG independently assessed their eligibility using an eligibility form based on the inclusion criteria. Each trial report was scrutinized to ensure data from a single trial with multiple publications were included only once. The trials' investigators were not contacted as eligibility was clear from the trial reports. There were no differences of opinion. We listed the excluded studies and the reasons for their exclusion.

Data extraction and management

SD and RB independently extracted data into pre-piloted data extraction forms designed by SD. We resolved any differences through discussion and contacted the corresponding publication author in the case of unclear or missing data. The number of individuals randomized to each treatment group and the number of individuals for whom outcomes were measured were extracted. We were unable to analyse data according to the intention-to-treat principle when there were losses to follow up. If there was discrepancy in the number randomized and the numbers analysed in each treatment group, we calculated the percentage loss to follow up and reported this information in a table.

For dichotomous outcome measures from controlled trials randomizing individuals, we recorded the number of participants experiencing the event and the number analysed in each treatment group. For continuous outcomes we did not extract arithmetic means and standard deviations, or medians and ranges as we had planned because this information was not presented. Data were reported using geometric means calculated to the base 10, and therefore we recorded this information and extracted the standard deviation on the log scale.

For trials randomized using clusters, we recorded the number of clusters in the trial, the average cluster size, and the unit of randomization. When the average sizes of clusters were not reported, we calculated them using the number of participants and the number of clusters. The statistical methods used to analyse the trial were documented along with details describing whether these methods adjusted for clustering or other covariates. We were unable to extract estimates of the intra-cluster correlation (ICC) coefficient for each outcome as they were not reported. When results were not adjusted for clustering, we extracted the same data as for the trials of individuals.

Assessment of risk of bias in included studies

SD and RB independently assessed the methodological quality of the trials and recorded the results in a table. We classified

generation of allocation sequence and allocation concealment as adequate, inadequate, or unclear according to [Jüni 2001](#). We reported who was blinded in each trial. We classified inclusion of all randomized participants as adequate if at least 90% of participants were followed up to the trial's completion; otherwise we classified inclusion as inadequate. We contacted the authors if this information was not specified or if it was unclear. We resolved any disagreements by discussion between review authors.

Data synthesis

SD entered data into [Review Manager 5](#). Each result is presented with a 95% confidence interval (CI).

Randomized controlled trials of individuals

We presented dichotomous data using risk ratios (RRs), and we compared continuous data reported as geometric means (calculated to the base 10) using geometric mean ratios (GMRs).

For dichotomous data, we planned to combine the data using RRs, but the data did not allow synthesis. We did not extract continuous data summarized by arithmetic means and standard deviations as we had intended and therefore we did not combine them using the mean differences (MDs). We did extract continuous data summarized using geometric means (to the base 10) and calculated GMRs, but we did not combine them on the log scale using the generic inverse variance method and report them on the anti-log scale as we had planned because the treatment comparisons varied across trials. Medians and ranges were not extracted (we had planned to report them in a table). We presented adverse event data in tables.

Cluster-randomized trials

We were unable to combine data adjusted for clustering with randomized trials of individuals using the generic inverse variance method as we had intended. When the results had not been adjusted for clustering, we conducted a series of sensitivity analyses in which we adjusted for the clustering by multiplying the standard errors of the estimates by the square root of the design effect; where the design effect is calculated as $DEff = 1 + (m - 1) \times ICC$, where m is the average cluster size. The ICC was unlikely to be reported, and so a range of reasonable values for the ICC were considered to observe how the results varied according to the value used.

Subgroup analysis and investigation of heterogeneity

Since no meta-analysis was carried out and there were too few trials, we did not investigate heterogeneity, conduct a sensitivity analysis to investigate the robustness of the results to the influence of quality components, or construct funnel plots to investigate publication bias.

As stated in the protocol, we intended to assess heterogeneity by inspecting the forest plots to detect overlapping confidence intervals, applying the χ^2 test with a P value of 0.10 indicating statistical significance, and also implementing the I^2 test with a value of 50% used to denote moderate levels of heterogeneity. We planned to use the random-effects model if heterogeneity was detected and it was still considered clinically meaningful to combine the trials. We aimed to explore potential sources of heterogeneity using the following subgroup analyses: variation in the age of the trial participants (children versus adults); type of

intervention (live versus attenuated); unit of randomization in the trial; and treatment regimen, dose, or route of administration. Additional sensitivity analyses were planned to investigate the robustness of the results to the influence of methodological quality components and to construct funnel plots to look for evidence of publication bias.

RESULTS

Description of studies

The search for relevant studies identified 11 trials, six of which were excluded because they were not randomized (see '[Characteristics of excluded studies](#)'). We also identified nine ongoing trials, which are summarized in the '[Characteristics of ongoing studies](#)'.

Included studies

Five trials were eligible for inclusion in the review (detailed in the '[Characteristics of included studies](#)'). [Burgasov 1976](#), a cluster-randomized trial, assessed the effect of the vaccine by cases of anthrax. The other four trials randomized individuals and were immunogenicity studies that assessed the effect of the different vaccine doses using changes in specific antibody titres ([Pittman 2002](#); [Gorse 2006](#); [Campbell 2007](#); [Marano 2008](#)).

Each trial had a different comparison: [Burgasov 1976](#) used STI vaccine (live-attenuated), while the other four compared different formulations, routes of administration, and schedules of a recombinant PA (rPA) vaccine and adsorbed anthrax vaccine (AVA) vaccine.

[Burgasov 1976](#) was conducted in the territory of the Chimkent and Dzhambul oblasts of the Kazakh Soviet Socialist Republic between 1973 and 1975. Two administration methods of the STI (live-attenuated) vaccine were compared against a control group receiving no vaccine. A total of 157,259 participants from 228 locations were enrolled; the trial included all inhabitants of anthrax-endemic localities aged over 14 years who had not been previously ill with anthrax and who had no medical contraindications to inoculations with STI vaccine. Forty-six per cent of the study participants were male. This cluster-randomized trial used stratified randomization to allocate 228 locations or clusters to one of three trial arms. A location contained up to 2000 persons, and stratification was based on average annual cases of anthrax and population size. The three trial arms were scarification (54,522 participants from 74 locations), needleless injection (52,763 participants from 77 locations), and no vaccine (49,974 participants from 77 locations).

[Pittman 2002](#) investigated a single lot (FAV032) of the United States AVA vaccine (Bioport Corporation, Lansing, MI), but there was no description of the trial's location or date. The trial aimed to compare six different abbreviated schedules and different routes of administration (subcutaneous or intramuscular) with the standard licensed regimen. [Pittman 2002](#) randomized 171 participants to seven treatment groups, each containing between 23 and 29 participants. Two additional military personnel were added to the control group, yielding a total of 173 participants. These two participants were not randomized, but it was not possible to separate out the results for these two non-randomized participants. Each participant received AVA (0.5 mL per dose) according to one of seven regimens: six groups received the vaccine subcutaneous or intramuscular at week zero, weeks zero and two, or weeks zero

and four; and the control group received the standard licensed regimen (subcutaneous doses at zero, two, and four weeks and six, 12, and 18 months). The participants were healthy volunteers aged between 18 and 64 years. Thirty-seven per cent of the volunteers (64/173) were female.

[Gorse 2006](#) was based at four study sites within the USA (trial date not stated). Two vaccines, a recombinant PA 102 (rPA102) vaccine and the USA version of AVA, and five treatment groups were included. The trial enrolled 100 healthy volunteers aged 18 to 40 years. Forty-one per cent (41/100) of the volunteers were female. Between 19 and 21 volunteers were assigned to each of the five treatment groups. Four were given rPA102 vaccine intramuscularly at zero, four, and eight weeks, with doses varying across the treatment groups (5, 25, 50, and 75 µg). The schedule was chosen empirically as this was the first clinical study of rPA102. The fifth group was given the USA version of AVA intramuscularly at weeks zero and four. Stratified randomization was used, with four groups of 25 volunteers each sequentially enrolled and randomized to the five treatment groups.

[Campbell 2007](#) was conducted at the Center for Vaccine Development at the University of Maryland School of Medicine, USA (date not stated). Participants received either AVA vaccine (BioThrax) or rPA vaccine (pilot lot manufactured by the biopharmaceutical development programme of the National Cancer Institute). Eighty healthy volunteers (exclusion criteria not reported) aged 18 to 40 years were enrolled; 31% (25/80) of the volunteers were female. Participants were randomly assigned to eight treatment groups, with 10 participants assigned to each regimen. Two treatment groups received AVA: the first received AVA subcutaneously on days zero, 14, and 28, and the second received AVA intramuscularly on days zero and 28. Six treatment groups received rPA vaccine with Alhydrogel adjuvant on days zero and 28, with doses varying across the treatment groups (5, 25, 50, and 75 µg). The remaining two treatment groups received rPA without Alhydrogel adjuvant on days zero and 28, with doses 5 and 25 µg.

[Marano 2008](#) was a non-inferiority trial that enrolled volunteers between May 2002 to February 2003 across study centres in the USA. In total, 1564 volunteers were randomized to seven treatment groups. The paper describes an interim report on the results at seven months for the first 1005 participants enrolled; hence this study is also listed as an ongoing study ([NCT00119067](#)). Two lots of AVA vaccine (BioThrax, Lansing, Michigan) were investigated, FAV063 and FAV 074. The trial aimed to compare the standard subcutaneous schedule with intramuscular administration given according to the same or an abbreviated schedule. Placebo groups receiving intramuscular or subcutaneous placebo injections were also included. Participants were randomized to six trial groups: subcutaneous AVA at weeks zero, two, and four and at month six; intramuscular AVA at weeks zero, two, and four and at month six; intramuscular AVA at weeks zero and four and at month six with placebo at week six (three groups were given this intervention); saline placebo at weeks zero, two, and four and at month six. The placebo group was randomized into a further two groups, the first group was given the vaccine by the intramuscular route and the second group by the subcutaneous route. Three intervention groups were given the same regimen up to month six (intramuscular AVA at weeks zero and four and at month six) and therefore these groups were combined in the analysis. The two placebo groups were also combined in the analysis. Vaccine and

placebo doses were administered as a 0.5 mL dose. The participants were healthy volunteers aged between 18 and 61 years, with two intact upper arms, who would comply with study procedures. Forty-nine per cent (500/1005) of the volunteers were female.

Outcome measures

One trial reported on cases of anthrax ("bacteriological or allergiological confirmation of the clinical diagnosis") from 25 days after and over the following two years ([Burgasov 1976](#)).

Four trials reported on various immunogenicity outcome measures, as measured by anti-PA antibodies and antibody seroconversion rates. [Pittman 2002](#) measured anti-PA IgG concentration (weekly for the first 12 weeks, then at weeks 14, 16, 18, 20, and 24) and antibody seroconversion rates at least once during the zero to eight weeks after the first AVA dose. [Gorse 2006](#) measured anti-PA antibodies one, two, and four weeks after each vaccination, and 12 and 24 weeks after the last vaccination. [Campbell 2007](#) measured anti-PA antibodies on the day of vaccination, and 14, 28, 42, 120, and 210 days after vaccination, and reported the results in a variety of outcomes: proportion of volunteers whose day 42 anti-PA IgG antibody concentration (ELISA) was greater than or equal to four times their day zero concentration and no less than 10 µg/mL; geometric mean concentration of anti-PA antibody on day 42; proportion of volunteers in each group with a four-fold increase in effective dilution 50 from day zero to day 42; and geometric mean titre of neutralizing antibodies measured by effective dilution 50 on day 42. [Marano 2008](#) measured anti-PA IgG at weeks zero, four and eight, and at months six and seven. The geometric mean concentration, the geometric mean titre, and the percentage of responders with a four-fold rise in titre, were reported at weeks six and eight, and at months six and seven. [Marano 2008](#) was a non-inferiority trial. Non-inferiority was achieved when the upper bound of the 95% CI for the ratio of the geometric means of the treatment group given subcutaneous AVA at weeks zero, two, and four and at month six, to that of the test groups was less than 1.5 and if the upper bound for the differences in proportions of four-fold response was less than 0.10 corresponding to a RR upper bound of less than 1.12.

All but [Burgasov 1976](#) reported on adverse reactions or adverse events, or both. [Pittman 2002](#) reported on systemic and local reactions identified by clinical evaluation of participants at 30 minutes, one to three days, one week, and one month after each vaccination. [Gorse 2006](#) reported on local and systemic reactogenicity. The data were collected using two methods: participants used a 14-day diary to record their presence or absence after each vaccination; and clinical laboratory assessments, including complete blood count, alanine aminotransferase, creatinine, and glucose, immediately prior to the first vaccination and one week after each vaccination. [Campbell 2007](#) collected data on both adverse reactions (local reactogenicity or systemic reactogenicity) and adverse events, including serious adverse events. Reactogenicity was determined using two methods: investigators observed participants for 30 minutes post-vaccination; and participants used a diary to record the presence of reactions and oral temperature on the day of the vaccine and the subsequent six days. The adverse events were observed for the duration of the study period. [Marano 2008](#) reported on local and systemic adverse events. Data were collected during scheduled examinations and from self-reported adverse event diaries, which were completed for 14 days after each of the

first two doses and for 28 days after each of the subsequent doses. Data were also collected from spontaneous reports made at any time during the study and through telephone follow-up calls to participants who did not return for scheduled visits. Data were presented for events reported during scheduled examinations. Examinations were done immediately before injection and 15 to 60 minutes and one to three days after each injection. Additional examinations were performed 28 days after injections three and four.

Analyses

[Burgasov 1976](#) carried out an available-case analysis (the analysis included data only from participants whose results were known).

[Pittman 2002](#) stated that an intention-to-treat analysis was performed by using all available data from all enrolled participants. However, the numbers in table 1 of the trial report indicate that IgG antibody test results were based on the 171 of the 173 participants available for follow up.

Similarly, [Gorse 2006](#) stated that an intention-to-treat analysis was performed, but the tabulated adverse effects results presented in the publication indicate that analyses were based on data only from participants whose results were known.

[Campbell 2007](#) stated that safety analyses "were performed for all enrolled volunteers at all time points at which data were collected" and "immunogenicity analysis was performed for all participants who received the vaccine as assigned".

[Marano 2008](#) stated that an intention-to-treat analysis was performed by imputing missing data using multiple imputation.

[Pittman 2002](#), [Gorse 2006](#), and [Marano 2008](#) calculated geometric means using a logarithmic scale with base 10. [Campbell 2007](#) did not specify how geometric means were calculated.

Risk of bias in included studies

See [Appendix 2](#) for a summary of the assessment and the 'Characteristics of included studies' for details of the methods used in each trial.

Generation of the allocation sequence

None of the included trials reported the method used to generate the randomization sequence; however, through correspondence we determined that [Campbell 2007](#) used computer generation to randomly assign volunteers to treatment groups (adequate method). [Pittman 2002](#) described the addition of two participants into the control group without using randomization techniques.

Allocation concealment

None of the trials described the method used to conceal the allocation of treatments.

Blinding

Treatment blinding procedures were not reported for [Burgasov 1976](#). Due to the nature of the intervention it is unlikely that either the study participants or those making a clinical diagnosis of anthrax were blinded.

In [Pittman 2002](#), the personnel conducting antibody assays were blinded to participants, treatments, and weeks of blood draw. However, as the study was open-label, the participants and those assessing adverse effects were not blinded.

In [Gorse 2006](#), participants and staff were blinded to the vaccine assignment until week eight, when the third immunization was administered and only then learned the assignment to AVA or rPA102.

Both volunteers and investigators were made unaware of the vaccine assignment in [Campbell 2007](#), except when the vaccine was given subcutaneously.

[Marano 2008](#) described blinding of the site investigators, persons involved in monitoring the adverse events, laboratory personnel, and statisticians. The staff involved in vaccine preparation and administration of the vaccine were not blinded. The participants were blinded with placebo.

Inclusion of all randomized participants followed up to the trial's completion

[Burgasov 1976](#) did not report losses to follow up. The number analysed was not reported so percentage calculations could not be made for this trial.

[Pittman 2002](#) followed up 171 out of 173 participants (99%) to the trial's completion (assessed as adequate). Reasons for loss to follow up were provided: one participant in the weeks zero and four intramuscular group was incarcerated for alleged automobile theft; and the second participant, who was in the control group, developed urticaria after the second dose of AVA. However, there is inconsistency between table 1 and the text of the publication regarding whether the lost participant from the control group actually belonged to the week zero intramuscular group.

In [Gorse 2006](#), 99% (99/100) of participants were followed up to the trial's completion (assessed as adequate). One volunteer in the 50 µg rPA102 group who discontinued the vaccination after the second dose was lost to follow up at week eight.

[Campbell 2007](#) did not specifically state whether volunteers were lost to follow up. However, seven of the 80 volunteers missed vaccinations and were reported as not included in the immunogenicity analysis. The data reported for local and systematic reactions appear to be based on the full 80 volunteers.

In the month seven interim-analysis, [Marano 2008](#) followed up 64.3% (1005/1564) of the participants randomized for inclusion. These were the first 1005 participants enrolled. In total 64 participants withdrew by month seven. The full data set will be unblinded in 2009, and more data should become available.

Effects of interventions

1. STI vaccine (different delivery): scarification and needleless vaccine vs no vaccine

[Burgasov 1976](#), a cluster-randomized trial, compared scarification and needleless vaccination methods with no vaccine.

1.1. Cases of anthrax

This trial only reported on the cases of anthrax, but the trial results were not adjusted for clustering and an estimate of the ICC coefficient could not be obtained from the publication.

With no adjustment for clustering, or assuming the ICC coefficient is zero, there is a statistically significant difference between cases of anthrax for the comparison of scarification versus no vaccine

(RR 0.25, 95% CI 0.09 to 0.69; 104,496 participants, 151 clusters) and needleless injection versus no vaccine (RR 0.16, 95% CI 0.05 to 0.54; 102,737 participants, 154 clusters). However, [Figure 1](#), which demonstrates the potential impact of the design effect for various ICC values, shows that even when the ICC is as small as 0.002 the results are no longer statistically significant. It is clear from [Figure 1](#) that with only small deviations from zero the ICC has a great influence on the CI widths.

Figure 1. The effect of clustering on the confidence interval of the risk ratio, for cases of anthrax, for the 2 comparisons (scarification vs no vaccine, needleless vs no vaccine) in [Burgasov 1976](#). The ICC is a measure of the amount of clustering in the data.

2. Adsorbed anthrax vaccine (AVA): different schedules and doses

[Pittman 2002](#) compared the current licensed schedule of AVA (given at weeks zero, two, and four) delivered subcutaneously with six groups with fewer AVA doses (given at week zero, weeks zero and two, or weeks zero and four) administered either intramuscularly or subcutaneously. [Marano 2008](#) compared a saline placebo with: subcutaneous AVA at weeks zero, two, and four and at month six; intramuscular AVA at weeks zero, two, and four and at month six; and intramuscular AVA at weeks zero and four and at month six.

2.1. Immunogenicity: anti-PA IgG antibodies

Anti-PA IgG concentrations

[Pittman 2002](#) showed that anti-PA IgG concentrations between weeks four and 24 were significantly higher in the licensed AVA schedule group (given at weeks zero, two, and four) than in the two groups that used fewer doses. The responses of the licensed AVA schedule group (given at weeks zero, two, and four) were higher at weeks four and five than the group that used doses at only weeks zero and four; however, no difference was observed from

week six to 24 (the trial authors only presented supporting results graphically).

The peak for the week zero groups was at three to four weeks; for the zero to two weeks groups at four to five weeks; and for the zero to four weeks groups and licensed AVA groups at six weeks. [Appendix 3](#) shows the geometric mean concentration (GMC) of anti-PA antibody at week six for all treatment groups and the geometric mean ratios for each treatment group compared to the control for [Pittman 2002](#). Results shows that, apart from the zero to four weeks subcutaneous group and the zero to four weeks intramuscular group, all groups were significantly inferior to the control group at week six ([Appendix 3](#)). When the zero to two weeks' and the zero to four weeks' schedules were compared by route of administration it was found that the groups given AVA four weeks apart had approximately three-fold or higher anti-PA IgG concentrations at peak compared to the groups given the vaccine two weeks apart (the trial authors only presented these results graphically).

[Marano 2008](#) reported the results for the anti-PA IgG concentrations at weeks four and eight and at months six and seven. The

geometric mean concentration and titres at week eight are presented in [Appendix 4](#). Measurements taken at week eight are presented because this time point was similar to the time that measurements were taken in the other included trials, and week eight was a focus of the trial report. Results showed that all interventions were significantly better than placebo. The four-dose intramuscular vaccine was not significantly different compared to the subcutaneous vaccine by our calculations at week eight (GMR 0.86, 95% CI 0.62 to 1.20). The three-dose intramuscular vaccine was significantly different at week eight to the four-dose intramuscular vaccine (GMR 0.57, 95% CI 0.42 to 0.78) and the subcutaneous vaccine (GMR 0.50, 95% CI 0.37 to 0.67) by our calculations. At month seven, all intramuscular AVA injection groups were non-inferior to the licensed subcutaneous regimen for all antibody responses. The geometric mean concentration for males and females in each treatment group was presented in the trial report in an investigation of the effect of gender on the outcome. At week eight, antibody responses were significantly higher in women than men with the four-dose intramuscular vaccine ($P = 0.002$ trial authors' calculations) and the three-dose intramuscular vaccine ($P = 0.003$ trial authors' calculations), but not for the subcutaneous vaccine. At month seven there was no difference between the genders in any group.

Seroconversion rates

For [Pittman 2002](#), [Appendix 3](#) shows the seroconversion rates and risk ratios compared to the licensed AVA schedule group (control group). Seroconversion rates were significantly lower for the single dose (week zero) groups, but they were above 95% in all other groups (see [Appendix 3](#)).

[Marano 2008](#) reported the four-fold rise in titre responses at weeks four and eight and at months six and seven. The percentage of participants with the four-fold rise in response, the incidence, and risk ratios for week eight are shown in [Appendix 4](#). Results show that all interventions were significantly better than placebo.

2.2. Adverse events

[Pittman 2002](#) reported on systemic and local reactions. Ten acute reactions were reported within the first 30 minutes after a total of 372 vaccine injections. All resolved and did not occur after a subsequent dose. No serious adverse reactions related to administration of AVA were reported.

Seven women became pregnant after completion of the AVA vaccination, one of whom had two pregnancies. All eight pregnancies resulted in healthy babies.

[Appendix 5](#) presents the systemic adverse event rates across all groups that administered the vaccine intramuscularly and across all groups that administered the vaccine by the subcutaneous route. Results are stratified for males and females as presented by the trial investigators. Systemic vaccine-related adverse reactions (see [Appendix 5](#)) recorded after each dose of AVA were reported by the trial authors to be independent of route, gender, or dose interval (but the trial report did not present any test of statistical significance). When compared to the licensed AVA schedule (control group), we found no significant differences in risk between groups based on an analysis subgrouped by gender.

Similarly, local adverse reactions are reported in [Appendix 7](#). Reactions such as subcutaneous nodules, erythema, induration,

and oedema were more common in women and associated with the subcutaneous route of administration (see [Appendix 7](#)) as stated in the trial report.

[Marano 2008](#) reported systemic adverse events for the four-dose intramuscular AVA vaccine and the subcutaneous vaccine. There were no significant differences between the vaccines for any adverse events ([Appendix 6](#)). The data presented were based on adverse events observed in-clinic; [Marano 2008](#) stated that results were consistent when adverse events reported by follow-up telephone calls and participants' diaries were included in the analysis. The trial's analysis showed that women had a greater odds of systemic adverse events (muscle ache, fatigue, and headache) compared to men.

[Marano 2008](#) reported local adverse events for the four-dose intramuscular AVA vaccine and the subcutaneous vaccine. There were significant differences between the vaccine routes for warmth, tenderness, itching, erythema, induration, oedema, and nodules ([Appendix 8](#)). The trial's analysis showed that the local reactions were of shorter duration in the intramuscular group compared to the subcutaneous group, and that participants in the intramuscular group experienced fewer moderate and severe local reactions (trial's analysis $P = 0.04$). The data presented were based on adverse events observed in-clinic; [Marano 2008](#) stated that results were similar when adverse events reported by follow-up telephone calls and participants' diaries were included in the analysis, with the exception of bruising (became significant) and pain (was reduced). Women were almost twice as likely as men to experience local reactions ($P < 0.01$) according to the trial's analysis.

During the first seven months of follow up, [Marano 2008](#) stated that there were 51 serious adverse events, including three deaths, in 47 participants. The data monitoring board concluded that no event was vaccine-related. In total, 229 serious adverse events in the entire study population (seven-month follow up available for 1005 at time of interim analysis) of 1563 participants were observed, including seven deaths. Causes of death were atherosclerotic cardiovascular disease, intracranial aneurysm, car accident, suicide, acquired immune deficiency syndrome (AIDS) related illness, accident, and gunshot wound. Nine events in seven participants were rated as possibly related to the study agent and were reported in the trial report.

3. rPA102 (different schedules) vs adsorbed anthrax vaccine (AVA)

[Gorse 2006](#) included five treatment groups: four were given rPA102 vaccine intramuscularly at zero, four, and eight weeks, with doses varying across the treatment groups (5, 25, 50, and 75 µg); and the fifth group was given the USA version of AVA intramuscularly at weeks zero and four.

3.1. Immunogenicity: anti-PA antibodies

The geometric mean antibody concentration and geometric mean titre for lethal toxin neutralization activity at week six (after two doses of either vaccine) are displayed in [Appendix 9](#) for all treatment groups.

Geometric mean titre

For the geometric mean titre, there was no statistically significant difference between the two vaccines except between AVA and the

50 µg rPA102 groups' mean titre (GMR 0.44, 95% CI 0.21 to 0.91; 100 participants).

Geometric mean antibody concentration

For geometric mean antibody concentration, differences were described as statistically significant for two comparisons: 50 µg rPA102 and AVA groups (GMR 0.15, 95% CI 0.06 to 0.39, 100 participants); and 75 µg rPA102 and AVA groups (GMR 0.26, 95% CI 0.12 to 0.56, 100 participants). The confidence intervals for some treatment groups could not be obtained from the trial report.

Seroconversion rates

Gorse 2006 did not present seroconversion rates.

3.2. Adverse events

Gorse 2006 reported on local and systemic reactogenicity.

The event rates for local reactions for the AVA group and for all rPA102 dose groups combined are shown in Appendix 10. Results are split by gender as presented by the trial investigators. Risk ratios are presented comparing event rates across AVA and rPA102 groups after each of the vaccine doses.

The event rates for systemic reactions are shown in Appendix 11. No clinically serious, dose-related toxicity or reactogenicity was observed. Local reactogenicity (mostly pain) was more common with AVA than rPA102 (see Appendix 10), while systemic reactogenicity (mostly headache) was more common among rPA102 participants, but only following the first vaccination (see Appendix 11). A greater proportion of women reported local and systemic reactogenicity than men. No significant differences in risk across the AVA and rPA groups was detected in a subgroup analysis based on gender.

4. rPA102 vs adsorbed anthrax vaccine (intramuscular or subcutaneous)

In Campbell 2007, two groups received AVA vaccine (intramuscularly on days zero and 28; or subcutaneously on days zero, 14, and 28), six groups received rPA102 vaccine with Alhydrogel adjuvant on days zero and 28 with doses varying across the treatment groups (5, 25, 50, and 75 µg), and the remaining two groups received rPA102 without Alhydrogel adjuvant on days zero and 28 (5 and 25 µg).

4.1. Immunogenicity

Appendix 12 presents the geometric mean concentration of anti-PA antibody responses at week six (day 42) and the geometric mean of neutralizing antibodies at week six. rPA102 vaccine, at a dose of 25 µg or 5 µg and without the Alhydrogel adjuvant, were statistically inferior to AVA given intramuscularly, based on geometric mean concentration and geometric mean titre (Appendix 12). The geometric mean concentration for the 25 µg dose group with Alhydrogel adjuvant was statistically significant (GMR 0.30, 95% CI 0.09 to 0.96; 20 participants, Appendix 12) compared to AVA given intramuscularly. The comparisons are based on a small number of participants.

Geometric mean antigen antibody responses were tabulated in the trial report at weeks zero, four, six, and 30. Single doses of vaccines led to little or no antibody responses, and antibody responses peaked at week six for all treatment groups. The highest

antibody response of the rPA vaccine groups was for the 50 µg dose group. Significant differences in peak responses were reported when comparing the rPA groups, with or without the Alhydrogel adjuvant, in the 5 µg dose group (GMC $P = 0.01$; GMT $P = 0.04$, trial authors' calculations) and the 25 µg dose group (GMC $P = 0.03$; GMT $P = 0.05$, trial authors' calculations). No dose response trend was observed across the rPA vaccine groups.

Seroconversion rates

Seroconversion rates were not presented quantitatively. It is reported that many volunteers who received rPA without adjuvant did not seroconvert and the 50 µg group had the highest proportion of seroconverters.

4.2. Adverse events

Campbell 2007 reported on adverse reactions (local and systemic reactogenicity) and adverse events (including serious adverse events). No volunteer suffered from an immediate reaction. Adverse reactions were reported for the week following each vaccination.

The proportion and frequency of local adverse reactions (tenderness and pain) based on the total number of vaccinees are shown in Appendix 13. rPA102 vaccine, at a dose of 25 µg or 5 µg and without the Alhydrogel adjuvant showed significantly lower risks of tenderness and pain compared to the AVA vaccine administered intramuscularly. Forearm swelling and lymphadenopathy were not observed during the follow-up period in any group. Six cases of erythema were observed in the AVA groups (five in the AVA subcutaneous and one in AVA intramuscular); swelling (three cases) and nodules (seven cases) were also observed in the AVA subcutaneous group.

The proportion of the frequency of systemic adverse reactions (anorexia and fatigue) of the total number of vaccinations are shown in Appendix 14. Due to the low event rates the confidence intervals for the risk ratios are extremely wide and therefore inferences cannot be made concerning significance. Fever under 39 °C was observed in the AVA subcutaneous group (two cases) and in the rPA 25 µg without Alhydrogel adjuvant group (one case). Fever over 39 °C was not observed.

No serious adverse events were observed; however unsolicited adverse events were reported as present (see Appendix 15). The definition of an unsolicited adverse event was not reported, although the trial report states that "about one-third were considered possibly, probably, or definitely vaccine related". The most common events were headaches and "mild respiratory complaints".

DISCUSSION

This review shows that limited data are available from randomized controlled trials on the clinical effectiveness, immunogenicity, and safety of anthrax vaccines. Five published randomized controlled trials met our inclusion criteria (159,176 participants).

Only one trial reported on the effectiveness of anthrax vaccine in protecting against the clinical disease (Burgasov 1976). This large field trial took place in the former USSR in areas where anthrax is endemic. The trial authors reported that a live-attenuated anthrax vaccine, STI, prevented 84% of cases when given by a needleless jet

injection and prevented 75% of cases when given by scarification, compared to an unvaccinated control group (Burgasov 1976). However, the trial had methodological limitations, in particular the investigators were not blind to the treatment allocation and the trial had a cluster-randomized design, but the authors did not perform a cluster-randomized analysis. We found that allowing for even a small amount of clustering resulted in no significant difference in the risk of anthrax cases between vaccinated and unvaccinated groups: however, the estimate of effect was large. The results should therefore be interpreted with caution.

We found no randomized controlled trials that investigated the ability of inactivated vaccines to protect against clinical disease. Four trials reported on the safety and immunogenicity (measured using anti-PA IgG antibodies) of inactivated vaccines, three of which were relatively small trials.

One trial compared four different regimens of AVA given subcutaneously with three regimens of AVA given intramuscularly (Pittman 2002). This trial found that giving two doses of the vaccine intramuscularly, separated by a four-week interval, had comparable immunogenicity to the licensed subcutaneous route. Intramuscular injection was safe and had a lower incidence of injection site reactions than subcutaneous injection. This trial had some methodological limitations including the addition of two participants to the control group without randomization.

Marano 2008 compared three regimens of AVA with placebo. The trial found that AVA given intramuscularly at weeks zero and four and at month six was not inferior to AVA given intramuscularly or subcutaneously at weeks zero, two, and four and at month six in terms of serum antibody responses at month seven. They found that AVA given at weeks zero, two, and four intramuscularly was not inferior to the subcutaneous vaccine given with the same schedule in terms of serum antibody responses at week eight or month seven. Intramuscular AVA at weeks zero and four was significantly inferior to the intramuscular and subcutaneous zero, two, and four week vaccine schedules in terms of serum antibody responses at week eight. All AVA regimens were significantly superior to placebo for immunogenicity response. Significantly fewer local adverse events were detected with the intramuscular compared to the subcutaneous route. The summary outcome data were only reported on the first 1005 participants (64% of the total) originally randomized. The full data set, which will include a 43-week follow up, will not be available until after the trial's completion in 2009.

Gorse 2006 performed a Phase I dose escalation trial to investigate the immunogenicity and safety of a recombinant vaccine, rPA102, compared to AVA. Three doses of the highest concentration of rPA102 induced a comparable titre of anti-PA IgG antibodies to AVA. rPA102 caused fewer local reactions, but resulted in more minor systemic adverse reactions than AVA. Neither vaccine produced any serious adverse effects. This trial had some methodological limitations: in particular AVA was not given according to the licensed route or schedule of administration.

Campbell 2007 performed a Phase I trial comparing four different doses of rPA with Alhydrogel adjuvant, and two different doses of rPA without Alhydrogel, against AVA given subcutaneously and AVA given intramuscularly. There were no significant reported differences between antibody concentrations in those given rPA vaccine with adjuvant at any dose compared to those given AVA by either route of administration. No dose response trend was observed across the rPA vaccine groups, when given with Alhydrogel. Those given rPA vaccine with Alhydrogel had significantly higher antibody responses than those given rPA without Alhydrogel, in both the 5 µg group and the 25 µg group. These trials show that rPA and AVA are safe and immunogenic. However, anti-PA IgG antibodies are only a surrogate marker of protection against anthrax and these trials do not prove that these inactivated vaccines can prevent clinical disease.

In summary, the trials included in this review provide limited evidence that vaccines for preventing anthrax are effective, immunogenic, and safe. In contrast, evidence from case reports, observational studies, and animal studies support the effectiveness, immunogenicity, and safety of anthrax vaccines (Jollenbeck 2002).

AUTHORS' CONCLUSIONS

Implications for practice

Based on data from five randomized controlled trials, there is evidence of protection against clinical anthrax for STI vaccine, although safety concerns with live vaccines mean specialists are more likely to recommend the AVA or a recombinant vaccine. There is currently insufficient evidence from the trials to compare the effects of AVA or rPA102.

Implications for research

The ongoing randomized controlled trials will provide further information on the immunogenicity and safety of different regimens of the currently available rPA102 and AVA. These trials will help to determine whether one or other vaccine is better and what the standard vaccination schedule should be. Randomized controlled trials to assess the effectiveness of these vaccines in protecting against anthrax disease are unlikely to be feasible because the disease is uncommon.

ACKNOWLEDGEMENTS

The authors are grateful to Tom Jefferson and colleagues for preparing the original review (Jefferson 1998).

This document is an output from a project funded by the UK Department for International Development (DFID) for the benefit of developing countries. The views expressed are not necessarily those of DFID.

REFERENCES

References to studies included in this review

Burgasov 1976 {published data only}

Burgasov P, Cherkasskij B, Knop A, Utegenov K. Epidemiological efficacy of the anthrax STI vaccine. *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii* 1976;**9**:27-35.

Campbell 2007 {published data only}

Campbell JD, Clement KH, Wasserman SS, Donegan S, Chrisley L, Kotloff KL. Safety, reactogenicity and immunogenicity of a recombinant protective antigen anthrax vaccine given to healthy adults. *Human Vaccines* 2007;**3**(5):205-11.

Gorse 2006 {published data only}

Gorse G, Keitel W, Keyserling H, Taylor D, Lock M, Alves K, et al. Immunogenicity and tolerance of ascending doses of a recombinant protective antigen (rPA102) anthrax vaccine: A randomized, double-blinded, controlled, multicenter trial. *Vaccine* 2006;**24**(33-34):5950-9.

Marano 2008 {published data only}

Marano N, Plikaytis BD, Martin SW, Rose C, Semenova VA, Martin SK, et al. Effects of a reduced dose schedule and intramuscular administration of anthrax vaccine adsorbed on immunogenicity and safety at 7 months: a randomized trial. *JAMA* 2008;**300**(13):1532-43.

Pittman 2002 {published data only}

Pittman P, Kim-Ahn G, Pifat D, Coonan K, Gibbs P, Little S, et al. Anthrax vaccine: immunogenicity and safety of a dose-reduction, route-change comparison study in humans. *Vaccine* 2002;**20**(9-10):1412-20.

References to studies excluded from this review

Brachman 1962 {published data only}

Brachman PS, Gold H, Plotkin SA, Fekety FR, Werrin M, Ingraham NR. Field evaluation of a human anthrax vaccine. *American Journal of Public Health* 1962;**52**(4):632-45.

Derbin 1981 {unpublished data only}

Derbin M, Sadovoj N, Tarumov V, Garin N. A comparative study of humoral anti-anthrax immunity in animals and humans immunized with live and chemical vaccines, UDK. Moscow: Central Scientific Research Institute of Epidemiology, 1981:1-7.

Grunow 2007 {published data only}

Grunow R, Porsch-Özcürümez M, Splettstoesser W, Buckendahl A, Hahn U, Beyer W. Monitoring of ELISA-reactive antibodies against anthrax protective antigen (PA), lethal factor (LF), and toxin-neutralising antibodies in serum of individuals vaccinated against anthrax with the PA-based UK anthrax vaccine. *Vaccine* 2007;**25**(18):3679-83.

Hepburn 2007 {published data only}

Hepburn M, Hugh Dyson E, Simpson A, Brennenman K, Bailey N, Wilkinson L, et al. Immune response to two different dosing

schedules of the anthrax vaccine precipitated (AVP) vaccine. *Vaccine* 2007;**25**(32):6089-97.

Shlyakhov 1994 {published data only}

Shlyakhov E, Rubinstein E. Delayed hypersensitivity after anthrax vaccination. II. Delayed hypersensitivity in humans vaccinated against anthrax [Hypersensibilite retardee charbonneuse post-vaccinale. II - Hypersensibilite retardee chez l'homme vaccine contre le charbon]. *Medecine Tropicale* 1994;**54**(2):133-6.

Stevtsov 2007 {published data only}

Shevtsov AN, Darmov IV, Saïtseva GA, Borovskoi DV, Menovshchikov VA, Kunof VK, et al. Assessment of immunity and allergy after vaccination with dry combined anthrax vaccine. *Zhurnal Mikrobiologii, Epidemiologii, i Immunobiologii* 2007;**5**:51-3.

References to ongoing studies

NCT00057525 {unpublished data only}

NCT00057525. A Phase 1 study of safety and immunogenicity of e. coli-derived recombinant protective antigen (rPA), a new anthrax vaccine administered by the intramuscular (IM) route in healthy adults. clinicaltrials.gov/ct2/show/NCT00057525 (accessed 12 January 2007).

NCT00063843 {unpublished data only}

NCT00063843. A Phase I, dose-escalating study to assess the safety, tolerability, and immunogenicity of recombinant anthrax protective antigen vaccine (rPA) administered in two intramuscular doses to healthy adults. clinicaltrials.gov/ct2/show/NCT00063843 (accessed 12 January 2007).

NCT00100724 {unpublished data only}

NCT00100724. A Phase 2 multi-center, randomized dose-finding trial of rPA-102 vaccine in healthy adult volunteers. clinicaltrials.gov/ct2/show/NCT00100724 (accessed 12 January 2007).

NCT00103467 {unpublished data only}

NCT00103467. A Phase 1 trial to evaluate the safety and immunogenicity of an anthrax recombinant protective antigen vaccine. clinicaltrials.gov/ct2/show/NCT00103467 (accessed 12 January 2007).

NCT00114621 {unpublished data only}

NCT00114621. Anthrax vaccine clinical trials. clinicaltrials.gov/ct2/show/NCT00114621 (accessed 12 January 2007).

NCT00119067 {unpublished data only}

Marano N, Babcock J, Keitel W, Keyserling H, Parker S, Poland P, et al. Interim analysis of anthrax vaccine human reactogenicity and immunogenicity clinical trial. Personal correspondence (email).

* NCT00119067. Anthrax vaccine adsorbed: human reactogenicity and immunogenicity trial to address change in

route of administration and dose reduction. clinicaltrials.gov/ct2/show/NCT00119067 (accessed 12 January 2007).

NCT00133484 {unpublished data only}

NCT00133484. A Phase II study to assess the safety, tolerability, immunogenicity, and optimal primary schedule of 3 recombinant protective antigen (rPA) anthrax vaccines administered in two intramuscular doses to healthy adults. clinicaltrials.gov/ct2/show/NCT00133484 (accessed 12 January 2007).

NCT00170456 {unpublished data only}

NCT00170456. A double-blind, parallel-group study of the safety, tolerability and immunogenicity of a range of doses and dosing schedules of Recombinant (rPA Based) Anthrax Vaccine in healthy subjects. clinicaltrials.gov/ct2/show/NCT00170456 (accessed 12 January 2007).

NCT00170469 {unpublished data only}

NCT00170469. A Phase II, dose ranging multi-centre, single blind, parallel-group, controlled study of the safety, tolerability and immunogenicity of recombinant (rPA based) anthrax vaccine compared with anthrax vaccine adsorbed in a healthy population. clinicaltrials.gov/ct2/show/NCT00170469 (accessed 12 January 2007).

Additional references

CDC 2006a

Centers for Disease Control and Prevention (CDC). What is the anthrax vaccine?. www.cdc.gov/ncidod/dbmd/diseaseinfo/anthrax_g.htm#whatisvacc (accessed 9 March 2006).

Dixon 1999

Dixon TC, Meselson M, Guillemin J, Hanna PC. Anthrax. *New England Journal of Medicine* 1999;**341**(11):815-26.

Inglesby 2002

Inglesby TV, O'Toole T, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, et al. Working Group on Civilian Biodefense. Anthrax as a biological weapon, 2002: updated recommendations for management. *JAMA* 2002;**287**(17):2236-52.

Jollenbeck 2002

Jollenbeck LM, Zwanziger LL, Durch JS, Strom BL, editors. The anthrax vaccine. Is it safe? Does it work?. Washington, DC: National Academy Press, 2002.

CHARACTERISTICS OF STUDIES

Characteristics of included studies [ordered by study ID]

Burgasov 1976

Methods

Design: cluster-randomized controlled trial

Generation of allocation sequence: not reported; stratified randomization by average annual anthrax morbidity level and population size of location

Allocation concealment: not reported

Jüni 2001

Jüni P, Altman DG, Egger M. Systematic reviews in health care: Assessing the quality of controlled clinical trials. *BMJ* 2001;**323**(7303):42-6.

Lefebvre 2008

Lefebvre C, Manheimer E, Glanville J. Chapter 6: Searching for studies. In: Higgins JPT, Green S (editors). *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.0.0 (updated February 2008). The Cochrane Collaboration, 2008. Available from www.cochrane-handbook.org.

Pitt 2001

Pitt ML, Little SF, Ivins BE, Fellows P, Barth J, Hewetson J, et al. In vitro correlate of immunity in a rabbit model of inhalational anthrax. *Vaccine* 2001;**19**(32):4768-73.

Reuveny 2001

Reuveny S, White MD, Adar YY, Kafri Y, Altboum Z, Gozes Y, et al. Search for correlates of protective immunity conferred by anthrax vaccine. *Infection and Immunity* 2001;**69**(5):2888-93.

Review Manager 5 [Computer program]

The Nordic Cochrane Centre, The Cochrane Collaboration. Review Manager (RevMan). Version 5.0. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008.

Salisbury 1996

Salisbury DM, Begg NT. Anthrax. Immunisation against infectious disease. London: HMSO, 1996:61-3.

References to other published versions of this review

Jefferson 1998

Jefferson T, Demicheli V, Deeks J, Graves P, Pratt M, Rivetti D. Vaccines for preventing anthrax. *Cochrane Database of Systematic Reviews* 1998, Issue 1. [DOI: [10.1002/14651858.CD000975.pub2](https://doi.org/10.1002/14651858.CD000975.pub2)]

Jefferson 2007

Jefferson T, Demicheli V, Deeks J, Graves P, Pratt M, Rivetti D. Vaccines for preventing anthrax. *Cochrane Database of Systematic Reviews* 2007, Issue 1. [DOI: [10.1002/14651858.CD000975](https://doi.org/10.1002/14651858.CD000975)]

* Indicates the major publication for the study

Burgasov 1976 (Continued)

Blinding: not reported

Inclusion of all randomized participants followed up to the trial's completion: not reported

Type of analysis: complete-case analysis; no adjustment for clustering

Participants	<p>Number: 228 clusters with 157,259 participants</p> <p>Inclusion criteria: age > 14 years without previous anthrax illness and no medical contra-indications to STI vaccine</p> <p>Exclusion criteria: not specified</p>
Interventions	<ol style="list-style-type: none"> 1. STI vaccine administered using scarification (54,522 participants, 74 locations) 2. STI vaccine administered by jet method (52,763 participants, 77 locations) 3. No vaccine (control) (49,974 participants, 77 locations)
Outcomes	<p>1. Anthrax-related morbidity</p> <p>Illnesses with anthrax occurring during the period beginning 25 days following immunization and continuing over the course of the following two years were recorded. Anthrax-related morbidity was recorded only in participants for whom it was possible to make a "bacteriological or allergiological confirmation of the clinical diagnosis". The details of how bacteriological and "allergiological" (presumably meaning serological) confirmation were obtained were not stated</p>
Notes	<p>Location: Chimkent and Dzhambul oblasts of the Kazakh Soviet Socialist Republic (former USSR)</p> <p>Date: 1973 to 1975</p> <p>Observation period after immunization: 25 days to 2 years</p> <p>Diagnosis: bacteriological or allergiological confirmation</p> <p>Other: investigations of whether the risk of morbidity was equally valued in each study group (in terms of exposure and animal morbidity) were carried out</p>

Campbell 2007

Methods	<p>Design: randomized controlled trial</p> <p>Generation of allocation sequence: computer generated (correspondence)</p> <p>Allocation concealment: not reported</p> <p>Blinding: neither the investigator nor the volunteers were made aware of the vaccine assignment</p> <p>Inclusion of all randomized participants followed up to the trial's completion: unclear; 7/80 volunteers missed vaccinations and were reported as not included in the immunogenicity analysis (4 volunteers in the recombinant protective antigen (rPA) vaccine groups missed 1 of the 2 vaccines, 2 volunteers in the adsorbed anthrax vaccine subcutaneous group missed 1 of the 3 vaccines, and 1 volunteer in the rPA vaccine 25 µg group was vaccinated on day 14 rather than day 28). The data reported for local and systematic reactions appears to be based on the full 80 volunteers</p> <p>Type of analysis: unclear</p>
Participants	<p>Number: 80</p> <p>Inclusion criteria: age 18 to 40 years; healthy volunteers</p> <p>Exclusion criteria: not specified</p>

Campbell 2007 (Continued)

Interventions	<p>rPA with Alhydrogel adjuvant was administered intramuscularly on days 0 and 28 at the following doses:</p> <ol style="list-style-type: none"> 1. 5 µg (10 participants) 2. 25 µg (10 participants) 3. 50 µg (10 participants) 4. 75 µg (10 participants) <p>rPA102 without Alhydrogel adjuvant was administered intramuscularly on days 0 and 28 at the following doses:</p> <ol style="list-style-type: none"> 5. 5 µg (10 participants) 6. 25 µg (10 participants) <ol style="list-style-type: none"> 7. Adsorbed anthrax vaccine on days 0 and 28 by intramuscular route (10 participants) 8. Adsorbed anthrax vaccine on days 0, 14, and 28 by subcutaneous route (10 participants)
Outcomes	<p>1. Anti-protective antigen antibodies Measured by toxin neutralization activity (TNA) and anti-protective antigen (PA) immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) on day of vaccination, and 14, 28, 42, 120, and 210 days after vaccination</p> <p><i>Note on data:</i> The report graphically presents the participant response (4-fold increase in concentration (using ELISA) or ED50 (using TNA), and the cumulative distribution of antibody responses</p> <p>1.1. Proportion of volunteers whose day 42 anti-PA IgG antibody concentration (ELISA) was ≥ 4 times their day 0 concentration and no less than 10 µg/mL</p> <p>1.2. Geometric mean concentration of anti-PA antibody on day 42</p> <p>1.3. Proportion of volunteers in each group with a 4-fold increase in effective dilution 50 from day 0 to day 42</p> <p>1.4. Geometric mean titre of neutralizing antibodies measured by effective dilution 50 on day 42 Effective dilution 50 is the reciprocal of the dilution of the sample that results in 50% neutralization of the lethal toxin</p> <p>2. Adverse reactions: local reactogenicity and system reactogenicity Classed as local reactogenicity or systemic reactogenicity. Volunteers were observed for 30 minutes post-vaccine by investigators to record any reactions. The volunteers used a diary to record the presence of reactions and oral temperature on the day of the vaccine and the subsequent 6 days</p> <p>Pain was classified as a local reaction and was measured using a pain assessment tool</p> <p>Local and systemic reactions were graded using a scale of 0 (no reaction) to 3 (severe reaction). Clinical laboratory assessments (complete blood count, chemistries, and liver and renal function tests) were collected immediately before vaccination, and 7 and 35 days after vaccination. The frequency and type of reactions are summarized for each treatment group. The mean pain score in the 30 minutes post-vaccine was reported for each treatment group and vaccine day</p> <p>3. Adverse events (including serious adverse events) Adverse events and serious adverse events were observed for the duration of the study period. The frequency of unsolicited and serious adverse events was reported</p>
Notes	<p>Location: Center for Vaccine Development at the University of Maryland School of Medicine (USA)</p> <p>Date: not given</p> <p>Observation period: antibody measurements were taken on the day of vaccination and 14, 28, 42, 120, and 210 days after vaccination. The volunteers used a diary to record the presence of reactions and oral temperature on the day of the vaccine and the subsequent 6 days</p> <p>Diagnosis: anti-PA antibodies were measured by TNA and anti-PA IgG ELISA</p>

Gorse 2006

Methods	<p>Design: multi-centre, randomized controlled trial; 4 groups of 25 volunteers each were sequentially enrolled and randomized</p> <p>Generation of allocation sequence: not reported; stratified by study site</p> <p>Allocation concealment: randomization sequence was generated by an outside collaborator (Statistics Collaborative, Washington, DC), but no further details provided</p> <p>Blinding: participants and trial staff volunteers were blinded to vaccine assignment until week 8, when the volunteers returned to the clinic for their potential third immunization</p> <p>Inclusion of all randomized participants followed up to the trial's completion: 99% (99/100); 2 volunteers in the 5 µg recombinant protective antigen 102 (rPA102) group discontinued the study after first dose but were followed up for 32 weeks – 1 due to an adverse reaction (urticarial rash) and 1 because of a "schedule conflict"; another volunteer in the 50 µg rPA102 group discontinued the vaccination after the second dose and was lost to follow up at week 8</p> <p>Type of analysis: intention-to-treat analysis stated; complete-case analysis presented</p>
Participants	<p>Number: 100</p> <p>Inclusion criteria: age 18 to 40 years; healthy volunteers</p> <p>Exclusion criteria: history of anthrax vaccination or exposure to <i>Bacillus anthracis</i>; human immunodeficiency virus (HIV-1), active hepatitis B or hepatitis C infection; history of immunodeficiency or malignant disease; use of immunosuppressive therapy within 5 years before study; and pregnancy</p>
Interventions	<p>rPA102 was administered intramuscular at 0, 4, and 8 weeks at the following doses:</p> <ol style="list-style-type: none"> 1. 5 µg (19 participants) 2. 25 µg (21 participants) 3. 50 µg (20 participants) 4. 75 µg (21 participants) <p>5. Adsorbed anthrax vaccine at weeks 0 and 4 by intramuscular route (19 participants)</p>
Outcomes	<p>1. Anti-protective antigen (PA) antibodies Measured by toxin neutralization activity (TNA) and anti-PA immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) on the day of vaccination; 1, 2, and 4 weeks after each vaccination; and 12 and 24 weeks after the last vaccination. Seroconversion was defined as a more than 4-fold increase in the minimum reactivity threshold for ELISA (2.5 µg/mL) or detectable TNA activity</p> <p><i>Note on data:</i> Report graphically presented the TNA geometric mean and anti-PA IgG ELISA geometric mean concentration by week and vaccine group. Comparison was made across all 5 treatment groups. However, as the AVA group received only 2 vaccinations compared with the 3 vaccinations in the rPA102 groups, the most pertinent time point for comparison with the AVA group is after the second dose of vaccine. Results concerning seroconversion were not presented</p> <p>2. Reactogenicity The volunteers used a 14-day diary to record the presence or absence of local and systemic reactogenicity after each vaccination. Clinical laboratory assessments, including complete blood count, alanine aminotransferase, creatinine, and glucose, were collected immediately before the first vaccination, and 1 week after each vaccination</p> <p><i>Note on data:</i> Frequency and duration of the reactions was summarized. The data across the 4 rPA102 groups were pooled and then compared to the AVA control group</p> <p>2.1. Local reactogenicity Local reactogenicity included redness, swelling, induration, bruising, warmth, pain, tenderness, and itching at the vaccination site; and limited arm motion, forearm swelling, and axillary lymph node swelling</p>

Gorse 2006 (Continued)

2.2. Systemic reactogenicity

Systemic reactogenicity included chills, nausea, vomiting, lack of appetite, difficulty breathing, muscle or body aches, joint pain, tiredness, headache, and rash

Notes

Location (4 study sites): St. Louis University Health Sciences Center (St. Louis); Department of International Health, Johns Hopkins University (Baltimore); Emory University School of Medicine (Atlanta); and Baylor College of Medicine (Houston)

Date: not given

Observation period: antibody measurements were taken on the day of vaccination; 1, 2, and 4 weeks after each vaccination; and 12 and 24 weeks after the last vaccination. The presence or absence of adverse effects were recorded in a 14-day diary after each vaccination

Diagnosis: anti-PA antibodies were measured by TNA and anti-PA IgG ELISA

Other: Comparison of outcomes is made across all 5 treatment groups for antibody response. The data across the 4 rPA102 groups regarding adverse events were pooled and then compared to the AVA control group

Marano 2008

Methods

Design: non-inferiority, randomized, double-blind, placebo-controlled trial

Generation of allocation sequence: unclear; randomization was stratified by gender and study site; at each study site a separate randomization sequence was used for each gender; block randomization was used to construct the randomization sequences using blocks of size 12

Allocation concealment: unclear; "Sex specific lists of participant identifiers were provided to the contract research organization (CRO), who made the study group assignments."; "The CRO provided participant identifiers with vaccine group assignments to unblinded individuals at the sites who then used these lists to sequentially assign participant identification numbers and study group assignments."

Blinding: staff involved in vaccine preparation and administration of the vaccine were not blinded; site investigators, persons involved in monitoring the adverse events, other staff, laboratory personnel, and statisticians were blinded; participants blinded with placebo

Inclusion of all randomized participants followed up to the trial's completion: the first 64.3% enrolled (1005/1564) followed up for the month 7 analysis; 64 withdraw from the study

Type of analysis: intention-to-treat analysis with imputed data

Participants

Number: 1564

Inclusion criteria: age 18 to 61 years; healthy volunteers with two intact upper arms who indicated willingness to comply with study procedures; females were eligible if they were not and did not plan to be pregnant

Exclusion criteria: history of anthrax infection or immunization against anthrax; known allergy to latex or vaccine preservatives; receiving experimental products, live or inactivated vaccine, immunosuppressive therapy, or immunoglobulin therapy within protocol-defined windows; active malignancy, cardiovascular disease, hepatic or renal insufficiency, current diabetes, or severe asthma; known infection with human immunodeficiency virus, hepatitis B, hepatitis C, or other conditions known to produce immune suppression; used high doses of inhaled steroids; neuropathy, unstable mental illness, or seizure disorder

Interventions

AVA administered under the following regimens:

1. Weeks 0, 2, and 4 and at 6 months; route subcutaneous (standard licensed version) (260 participants)
2. Weeks 0, 2, and 4 and at 6 months; route intramuscular (262 participants)

Marano 2008 (Continued)

3. Weeks 0 and 4; and at 6 months; route intramuscular; placebo received at week 2 (256 participants)
4. Weeks 0 and 4 and at 6 months; route intramuscular; placebo received at week 2 (258 participants)
5. Weeks 0 and 4 and at 6 months; route intramuscular; placebo received at week 2 (268 participants)
6. Placebo (saline) intramuscular at weeks 0, 2, and 4 and at 6 months (127 participants)
7. Placebo (saline) subcutaneous at weeks 0, 2, and 4 and at 6 months (133 participants)

Vaccine and placebo administered as a 0.5 mL dose

Outcomes

1. Immunogenicity

Immune response to AVA was determined by assay of anti-protective antigen (PA) immunoglobulin G (IgG) antibody specific for *Bacillus anthracis* by using a validated enzyme-linked immunosorbent assay (ELISA). Serum samples were drawn at weeks 0, 4, and 8 and months 6 and 7 to be assayed; results reported at each of these time points

1.1. Anti-PA IgG antibody geometric mean concentration

The limit of detection of the assay was 25 µg/mL. Antibody concentrations that were below the assay reactivity thresholds were reported as 1.25 µg/mL

1.2. Geometric mean titre

The limit of detection of the assay was 50 for titres. Antibody titres that were below the assay reactivity thresholds were reported as 1/25 respectively.

1.3. Proportion of responders with a 4-fold rise in titre

2. Adverse events (including serious adverse events)

An adverse event was defined as any untoward medical occurrence, regardless of causal relationship to vaccination. Data were collected during scheduled examinations, self-reported using adverse event diaries, and spontaneously reported at any time during the study and through follow-up by telephone of participants who did not return for scheduled visits. The results were presented for events reported during scheduled examinations. Examinations were done immediately before injection and 15 to 60 minutes and 1 to 3 days after injection. Additional examinations were performed 28 days after injections 3 and 4

Participants recorded adverse events in diaries for 14 days after each of the first 2 doses and for 28 days after all subsequent doses. Pain defined as a subjective feeling of discomfort in the area of the injection site; this is not pain on injection, which is assessed immediately following the injection using a visual scale. The following events were classified as serious: death; life-threatening event; initial inpatient hospitalization or prolongation of hospitalization; significant or persistent disability or incapacity; congenital anomaly or birth defect; and a medical event that required medical or surgical intervention to prevent one of the other outcomes

Notes

Location: USA; study centres included the Walter Reed Army Institute of Research, Maryland; Baylor College of Medicine, Texas; Emory University School of Medicine, Georgia; Mayo Clinic, Minnesota; and University of Alabama at Birmingham

Date: enrolled May 2002 to February 2003

Observation period after immunization: followed up for 60 days beyond the last scheduled injection

Diagnosis: anti-PA antibodies were measured by anti-PA IgG ELISA

Trial registration number: NCT00133484

Pittman 2002

Methods

Design: randomized, open-label, controlled trial

Generation of allocation sequence: not reported

Allocation concealment: not reported

Pittman 2002 (Continued)

Blinding: single-blind; personnel conducting antibody assays were blinded to participants, treatments, and weeks of blood draw

Inclusion of all randomized participants followed up to the trial's completion: 98.84% (171/173) followed up; 2 participants did not complete the initial series (1 in the 0 to 4 weeks intramuscular group was incarcerated for alleged auto theft; the second drop out was in the control group (although there is inconsistency in the paper regarding this) and developed urticaria after the second dose of adsorbed anthrax vaccine (AVA))

Type of analysis: intention-to-treat analysis stated; complete-case analysis presented

Participants	<p>Number: 171 participants randomized; another 2 added to the control group</p> <p>Inclusion criteria: not specified</p> <p>Exclusion criteria: pregnant; human immunodeficiency virus (HIV) positive; or acutely ill with an oral temperature $\geq 38.3^{\circ}\text{C}$</p>
Interventions	<p>AVA administered under the following regimens:</p> <ol style="list-style-type: none"> 1. Week 0; route subcutaneous (25 participants) 2. Week 0; route intramuscular (25 participants) 3. Weeks 0 and 2; route subcutaneous (25 participants) 4. Weeks 0 and 2; route intramuscular (25 participants) 5. Weeks 0 and 4; route subcutaneous (23 participants) 6. Weeks 0 and 4; route intramuscular (22 participants) 7. Weeks 0, 2, and 4; route subcutaneous (control/standard licensed version) (28 participants)
Outcomes	<p>1. Immunogenicity</p> <p>Immune response to AVA was determined by assay of anti-protective antigen (PA) immunoglobulin G (IgG) antibody specific for <i>Bacillus anthracis</i> by using a validated enzyme-linked immunosorbent assay (ELISA) developed for this study. The overall cumulative rates of antibody response were determined based on a positive response at least once up to 8 weeks after the first dose. The limit of detection of the assay was 25 $\mu\text{g/mL}$; IgG concentration $> 25 \mu\text{g/mL}$ considered a positive response</p> <p>1.1. Anti-PA IgG concentration</p> <p>Measured using 15 mL blood samples. The samples were drawn from each participant at scheduled intervals (weekly for the first 12 weeks, then at weeks 14, 16, 18, 20, and 24)</p> <p>Anti-PA IgG concentrations measured at peak for each schedule were summarized using geometric means. The peak for the 0 week groups was at 3 to 4 weeks; for the 0 and 2 weeks groups at 4 to 5 weeks; and for the 0 and 4 weeks groups and control groups at 6 weeks</p> <p>1.2. Antibody seroconversion rates</p> <p>Defined as the percentage of volunteers exceeding detectable anti-PA IgG concentration ($> 25 \mu\text{g/mL}$) at least once during the 0 to 8 weeks after the first AVA dose</p> <p>2. Systemic and local reactions</p> <p>Clinical evaluation of participants for local and systemic reactions was carried out at 30 minutes, 1 to 3 days, 1 week, and 1 month after each vaccination. The reactions were observed to be present or absent, measured for maximum dimension, or graded for severity. Local reactions were defined as signs and symptoms at the injection site, such as erythema, induration, swelling, pain, pruritis, etc. Systemic reactions were defined as all reactions not at the injection site itself</p> <p>In addition to comparisons of local and systemic reactions between treatment arms, the trial aimed to discern if females had a higher rate of reactions to the vaccine than men. All systemic and local reactions were recorded and tabulated separately for males and females in intramuscular and subcutaneous injection groups</p>
Notes	<p>Location: not given</p> <p>Date: not given</p>

Pittman 2002 (Continued)

Observation period after immunization: examined for reactions at 30 minutes, 1 to 3 days, 1 week, and 1 month after each vaccine

Diagnosis: anti-protective antigen antibodies were measured by anti-PA IgG G ELISA

Characteristics of excluded studies [ordered by study ID]

Study	Reason for exclusion
Brachman 1962	Not a randomized trial
Derbin 1981	Not a randomized trial
Grunow 2007	Not a randomized trial
Hepburn 2007	Not a randomized trial
Shlyakhov 1994	Not a randomized trial
Stevtsov 2007	Not a randomized trial

Characteristics of ongoing studies [ordered by study ID]

NCT00057525

Trial name or title	"A Phase 1 Study of Safety and Immunogenicity of <i>E. coli</i> -Derived Recombinant Protective Antigen (rPA), a New Anthrax Vaccine Administered by the Intramuscular (IM) Route in Healthy Adults"
Methods	Randomized, double-blind, placebo controlled trial
Participants	Eligibility criteria: healthy volunteers; male/female; 18 to 40 years Expected enrolment: 70
Interventions	rPA intramuscular injection at time 0 and 1 month Doses will range from 5 to 100 µg Combined with phosphate-buffered saline or adsorbed to Alhydrogel
Outcomes	1. Safety 2. Immunogenicity Follow up: not given
Starting date	Study start: April 2003 Expected completion: April 2004 Last follow up: not given Data entry closure: not given Trial information source last updated: 23 June 2005

NCT00057525 (Continued)

Contact information	MJ Humphries (mjhumphries@hivresearch.org)
Notes	Location: Maryland, USA Registration number: NCT00057525 Sponsors and collaborators: DynPort Vaccine Company

NCT00063843

Trial name or title	"A Phase I, Dose-Escalating Study to Assess the Safety, Tolerability, and Immunogenicity of Re-combinant Anthrax Protective Antigen Vaccine (rPA) Administered in Two Intramuscular Doses to Healthy Adults"
Methods	Randomized, double-blind, active control trial
Participants	Eligibility criteria: healthy volunteers; male/female; 18 to 40 years Expected enrolment: 80
Interventions	1. rPA vaccine: 2 intramuscular injections in presence and absence of Alhydrogel (given 1 month apart) 2. Anthrax vaccine adsorbed given by either the intramuscular or subcutaneous route in 3 doses at 24-day intervals
Outcomes	1. Safety 2. Tolerability 3. Immunogenicity Follow up: 210 days
Starting date	Study start: July 2003 Expected completion: August 2005 Last follow up: August 2005 Data entry closure: not given Information source last updated: 26 May 2006
Contact information	Not given
Notes	Location: Maryland, USA Sponsors and collaborators: National Institute of Allergy and Infectious Diseases (NIAID)

NCT00100724

Trial name or title	"A Phase 2 Multi-Center, Randomised Dose-Finding Trial of rPA-102 Vaccine in Healthy Adult Volunteers"
Methods	Randomized, double-blind trial
Participants	Eligibility criteria: healthy volunteers; male/female; 18 to 55 years

NCT00100724 (Continued)

Expected enrolment: 480

Interventions	Recombinant protective antigen 102 (rPA102) intramuscular injection: 0 and 4 weeks; 2 dose ranges, and 4 adjuvant levels
Outcomes	1. Safety 2. Immunogenicity Follow up: not given
Starting date	Study start: April 2004 Expected completion: not given Last follow up: July 2005 Data entry closure: September 2005 Information source last updated: 10 January 2006
Contact information	Not given
Notes	Locations: 12 locations in the USA Sponsors and collaborators: VaxGen; National Institutes of Health (NIH); National Institute of Allergy and Infectious Diseases (NIAID); Centers for Disease Control and Prevention

NCT00103467

Trial name or title	"A Phase 1 Trial to Evaluate the Safety and Immunogenicity of an Anthrax Recombinant Protective Antigen Vaccine"
Methods	Randomized, double-blind, active control trial
Participants	Eligibility criteria: male/female; 18 to 40 years Expected enrolment: 100
Interventions	1. Recombinant protective antigen 102 (rPA102) vaccine: 3 injections at 4 different doses 2. Anthrax vaccine adsorbed
Outcomes	1. Safety 2. Immunogenicity Follow up: not given
Starting date	Study start: June 2003 Expected completion: not given Last follow up: not given Data entry closure: not given Information source last updated: 23 June 2005
Contact information	Not given
Notes	Locations: 4 locations in the USA

Vaccines for preventing anthrax (Review)

NCT00103467 (Continued)

Sponsors and collaborators: VaxGen

NCT00114621

Trial name or title	"Anthrax Vaccine Clinical Trials"
Methods	Randomized trial
Participants	Eligibility criteria: healthy male/females; 18 to 45 years Expected enrolment: 300 to 350
Interventions	1. Recombinant protective antigen (rPA) vaccine in 2 different doses (10 µg and 20 µg) 2. Anthrax vaccine adsorbed Vaccine administered at 0, 2, and 4 months, and at 1 year
Outcomes	1. Safety 2. Immunogenicity Follow up: not given
Starting date	Study start: September 2004 Expected completion: not given Last follow up: not given Data entry closure: not given Information last updated: 12 December 2006
Contact information	Patient Recruitment and Public Liaison Office (prpl@mail.cc.nih.gov)
Notes	Locations: 6 locations in the USA Sponsors and collaborators: National Institute of Child Health and Human Development (NICHD)

NCT00119067

Trial name or title	"Anthrax Vaccine Adsorbed: Human Reactogenicity and Immunogenicity Trial to Address Change in Route of Administration and Dose Reduction"
Methods	Randomized, double-blind, placebo control, bio-equivalence study
Participants	Eligibility criteria: healthy male/female; 18 to 61 years Expected enrolment: 1564
Interventions	1. Adsorbed anthrax vaccine: 5 different regimens (1 subcutaneous and 4 different intramuscular mL regimens) 2. Placebo
Outcomes	1. Safety 2. Immunogenicity

NCT00119067 (Continued)

Follow up: 25 visits

Starting date	Study start: May 2002 Expected completion: second quarter of 2009 Last follow up: December 2007 Data entry closure: March 2008 Information source last updated: 3 August 2005
Contact information	Not given
Notes	Preliminary results from follow up of the first 1005 participants for 7 months have been reported in Marano 2008 (included study) Locations: 5 locations in the USA Sponsors and collaborators: Centers for Disease Control and Prevention; Walter Reed Army Institute of Research (WRAIR); Baylor College of Medicine; University of Alabama at Birmingham; Emory University; Mayo Clinic

NCT00133484

Trial name or title	"A Phase II Study to Assess the Safety, Tolerability, Immunogenicity, and Optimal Primary Schedule of 3 Recombinant Protective Antigen (rPA) Anthrax Vaccines Administered in Two Intramuscular Doses to Healthy Adults"
Methods	Randomized, double-blind, active control trial
Participants	Eligibility criteria: healthy adults; male/female; 18 to 50 years Expected enrolment: 270
Interventions	1. 3 recombinant protective antigen (rPA) vaccines administered at 2 intramuscular dose regimens of 100 µg rPA 2. Anthrax vaccine adsorbed administered as subcutaneous 3 doses Over a 1-week to 4-week period
Outcomes	1. Safety 2. Tolerability 3. Immunogenicity 4. Long-term immune response (for a subset) Follow up: 12 months (12 to 14 follow-up visits) after a 30-day screening period
Starting date	Start date: not given Expected completion: May 2007 Last follow up: not given Data entry closure: not given Information source last updated: 4 May 2006
Contact information	James Campbell (jcampbel@medicine.umaryland.edu)

NCT00133484 (Continued)

Notes

Locations: 2 locations in the USA

Sponsors and collaborators: National Institute of Allergy and Infectious Diseases (NIAID)

NCT00170456

Trial name or title	"A Double-Blind, Parallel-Group Study of the Safety, Tolerability and Immunogenicity of a Range of Doses and Dosing Schedules of Recombinant (rPA Based) Anthrax Vaccine in Healthy Subjects"
Methods	Randomized, double-blind, placebo control trial
Participants	Eligibility criteria: healthy male/female; 18 to 55 years Expected enrolment: 400
Interventions	rPA injection with Alhydrogel with different vaccine schedules
Outcomes	1. Safety 2. Tolerability 3. Immunogenicity Follow up: not given
Starting date	Study start: March 2005 Expected completion: February 2007 Last follow up: not given Data entry closure: not given Information source last updated: 3 November 2005
Contact information	Not given
Notes	Locations: 2 locations in the United Kingdom Sponsors and collaborators: National Institute of Allergy and Infectious Diseases (NIAID)

NCT00170469

Trial name or title	"A Phase II, Dose Ranging Multi-Centre, Single Blind, Parallel-Group, Controlled Study of the Safety, Tolerability and Immunogenicity of Recombinant (rPA Based) Anthrax Vaccine Compared With Anthrax Vaccine Adsorbed in a Healthy Population"
Methods	Randomized, single-blind trial
Participants	Eligibility criteria: healthy male/female; 18 to 55 years Expected enrolment: 200
Interventions	1. rPA anthrax vaccine over a range of doses 2. Anthrax vaccine adsorbed
Outcomes	1. Safety 2. Tolerability

Vaccines for preventing anthrax (Review)

NCT00170469 (Continued)

3. Immunogenicity

Follow up: not given

Starting date	Study start: March 2005 Expected completion: January 2007 Last follow up: not given Data entry closure: not given Information source last updated: 24 August 2006
Contact information	Not given
Notes	Locations: 16 locations in the USA Sponsors and collaborators: National Institute of Allergy and Infectious Diseases (NIAID)

APPENDICES

Appendix 1. Search methods: detailed search strategies

Search set	CIDG SR ^a	CENTRAL	MEDLINE ^b	EMBASE ^b	LILACS ^b	SCI
1	anthrax	ANTHRAX	ANTHRAX	ANTHRAX	anthrax	anthrax
2	vaccin*	anthrax	anthrax	anthrax	vaccin*	Bacillus anthracis
3	1 and 2	Bacillus anthracis	Bacillus anthracis	Bacillus anthracis	1 and 2	vaccine
4	—	1 or 2 or 3	1 or 2 or 3	1 or 2 or 3	—	1 and 2
5	—	vaccin*	vaccin*	vaccin\$	—	3 and 4
6	—	4 and 5	4 and 5	4 and 5	—	AVA
7	—	AVA	AVA	ANTHRAX-VACCINE	—	5 or 6
8	—	6 or 7	6 or 7	6 or 7	—	—
9	—	—	Limit 8 to human	AVA	—	—
10	—	—	—	8 or 9	—	—
11	—	—	—	Limit 10 to human	—	—

^aCochrane Infectious Diseases Group Specialized Register.

^bSearch terms used in combination with the search strategy for retrieving trials developed by The Cochrane Collaboration ([Lefebvre 2008](#)); upper case: MeSH or Emtree heading; lower case: free text term.

Appendix 2. Risk of bias assessment^a

Trial	Allocation sequence generation	Allocation concealment	Blinding	Inclusion ^b (n/N, %) ^c
Burgasov 1976	Unclear	Unclear	Unclear	Unclear
Campbell 2007	Adequate	Unclear	Investigator and volunteers blinded	Adequate (73/80, 91%)
Gorse 2006	Unclear	Unclear	Participants and trial staff volunteers were blinded to vaccine assignment until week 8, when the volunteers returned to the clinic for their potential third immunization	Adequate (99/100, 99%)
Marano 2008	Unclear	Unclear	Participants blinded; staff involved in vaccine preparation and administration of the vaccine were not blinded; site investigators, persons involved in monitoring the adverse events, laboratory personnel, and statisticians were blinded	Inadequate (1005/1564, 64.3%)
Pittman 2002	Unclear	Unclear	Single blind (personnel conducting antibody assays)	Adequate (171/173, 99%)

^aDetails of each method available in the '[Characteristics of included studies](#)'.

^bInclusion of all randomized participants followed up to the trial's completion.

^cn: number of participants followed up; N: number of participants randomized; %: percentage of randomized participants that were followed up.

Appendix 3. AVA (different schedules and doses): immunogenicity (anti-protective antigen antibody)^a

Schedule	Geometric mean concentration (µg/mL) (95% CI) ^b	Geometric mean ratio (95% CI)	Seroconversion rate (%)	Risk ratio (95% CI)
Weeks 0, 2, and 4 subcutaneous (control)	478 (313 to 728)	—	28/28 (100)	—
Week 0 subcutaneous	36 (22 to 58)	0.08 (0.04 to 0.14)	14/25 (56)	0.56 (0.40 to 0.79)
Week 0 intramuscular	18 (15 to 22)	0.04 (0.02 to 0.06)	7/24 (29)	0.29 (0.16 to 0.54)
Weeks 0 and 2 subcutaneous	205 (132 to 320)	0.43 (0.23 to 0.79)	25/25 (100)	Not estimable
Weeks 0 and 2 intramuscular	147 (89 to 245)	0.31 (0.16 to 0.59)	24/25 (96)	0.96 (0.89 to 1.04)
Weeks 0 and 4 subcutaneous	625 (404 to 971)	1.31 (0.71 to 2.40)	23/23 (100)	Not estimable

(Continued)

Weeks 0 and 4 intramuscular	482 (304 to 764)	1.01 (0.54 to 1.88)	20/21 (95)	0.95 (0.87 to 1.05)
-----------------------------	------------------	---------------------	------------	---------------------

Data source: [Pittman 2002](#).

AVA: adsorbed anthrax vaccine; CI: confidence interval.

^aMeasured at week 6.

^bGeometric means to the base 10.

Appendix 4. AVA (different schedules and doses)^a: immunogenicity (anti-protective antigen antibody)

Schedule	Geometric mean concentration ^{a,b}		Geometric mean titre ^a		Responders with 4-fold rise in titre			
	µg/mL (95% CI)	Geometric mean ratio (95% CI)	95% CI	Geometric mean ratio (95% CI)	Percentage (95% CI) (no. participants)	Incidence	Risk ratio (95% CI)	Risk ratio (non-event) ^c (95% CI)
AVA (subcutaneous) at weeks 0, 2, 4 and month 6	105.1 (83.8 to 131.8)	79.02 (62.32 to 100.19)	1315.4 (1047.8 to 1651.4)	50.59 (39.99 to 64.00)	98.8 (95.2 to 99.9) (165 participants)	163/165	166.95 (23.65 to 1178.42)	0.01 (0.00 to 0.05)
AVA (intramuscular) at weeks 0, 2, 4 and month 6	90.8 (71.9 to 114.6)	68.27 (53.50 to 87.11)	1114.8 (881.9 to 1409.3)	42.88 (33.67 to 54.61)	97.7 (93.5 to 99.5) (170 participants)	166/170	165.02 (23.38 to 1164.89)	0.02 (0.01 to 0.06)
AVA (intramuscular) at weeks 0 and 4 and month 6	52.2 (42.6 to 63.9)	39.25 (31.66 to 48.66)	650.6 (533.3 to 793.7)	25.02 (20.33 to 30.79)	94.4 (91.6 to 96.5) (501 participants)	472/501	159.22 (22.56 to 1123.89)	0.06 (0.04 to 0.08)
Placebo at weeks 0, 2, 4, and month 6	1.33 (1.24 to 1.43)	—	26.0 (24.5 to 27.6)	—	0.6 (0.01 to 3.7) (169 participants)	1/169	—	—

Data source: [Marano 2008](#).

AVA: adsorbed anthrax vaccine; CI: confidence interval.

^aAt week 8. Geometric means to the base 10.

^bAnti-protective antigen.

^cSwopped the event and non-event, ie risk of not showing a 4-fold rise in titre.

Appendix 5. AVA (different schedules and doses): systemic adverse reactions^a

Reaction	Gender	Incidence (%) ^b		Risk ratio (95% CI)
		Intramuscular	Subcutaneous	
Headache	Female	8/46 (17)	8/71 (11)	1.54 (0.62 to 3.82)
	Male	5/72 (7)	12/132 (9)	0.76 (0.28 to 2.08)
Anorexia	Female	4/46 (9)	0/71 (0)	12.35 (0.67 to 228.14)
	Male	2/72 (3)	3/132 (2)	1.22 (0.21 to 7.15)
Malaise	Female	3/46 (7)	6/71 (9)	0.77 (0.20 to 2.93)
	Male	3/72 (4)	13/132 (10)	0.42 (0.12 to 1.44)
Myalgia	Female	1/46 (2)	5/71 (7)	0.31 (0.04 to 2.56)
	Male	4/72 (6)	4/132 (3)	1.83 (0.47 to 7.11)
Nausea	Female	3/46 (7)	2/71 (3)	2.32 (0.40 to 13.33)
	Male	2/72 (3)	3/132 (2)	1.22 (0.21 to 7.15)
Respiratory difficulty	Female	1/46 (2)	1/71 (1)	1.54 (0.10 to 24.07)
	Male	3/72 (4)	4/132 (3)	1.38 (0.32 to 5.98)
General pruritus	Female	0/46 (0)	2/71 (3)	0.39 (0.02 to 8.37)
	Male	0/72 (0)	3/132 (2)	0.31 (0.02 to 6.02)
Fever	Female	0/46 (0)	1/71 (1)	0.77 (0.03 to 22.54)
	Male	1/72 (1)	4/132 (3)	0.46 (0.05 to 4.02)

Data source: [Pittman 2002](#).

AVA: adsorbed anthrax vaccine; CI: confidence interval.

^aData measured after 1 to 3 doses of AVA. We combined data by administration route: intramuscular and subcutaneous.

^bThe denominator is the number of doses administered.

Appendix 6. AVA (at weeks 0, 2, and 4): systemic reactogenicity^a

Reaction	Gender	Incidence (%) ^b	Risk ratio (95% CI)
----------	--------	----------------------------	---------------------

(Continued)

		AVA intramuscular	AVA subcutaneous	
Fatigue	Female	40/336 (11.9)	32/310 (10.3)	1.15 (0.74 to 1.79)
	Male	20/302 (6.6)	19/319 (6.0)	1.11 (0.61 to 2.04)
Muscle ache	Female	32/335 (9.6)	22/310 (7.1)	1.35 (0.80 to 2.26)
	Male	21/302 (7.0)	12/320 (3.8)	1.85 (0.93 to 3.70)
Headache	Female	28/335 (8.4)	33/310 (10.6)	0.79 (0.49 to 1.27)
	Male	12/302 (4.0)	14/319 (4.4)	0.91 (0.43 to 1.93)
Fever	Female	0/331 (0)	0/309 (0)	Not estimable
	Male	0/300 (0)	0/319 (0)	Not estimable
Tender/painful axillary adenopathy	Female	2/334 (0.6)	8/309 (2.6)	0.23 (0.05 to 1.08)
	Male	1/300 (0.3)	3/319 (0.9)	0.35 (0.04 to 3.39)

Data source: [Marano 2008](#).

AVA: adsorbed anthrax vaccine; CI: confidence interval.

^a During clinic examinations.

^b Denominator is number of doses administered.

Appendix 7. AVA (different schedules and doses): local adverse reactions^a

Reaction	Gender	Incidence (%) ^b		Risk ratio (95% CI)
		Intramuscular	Subcutaneous	
Tenderness	Female	31/46 (67)	60/71 (85)	0.80 (0.64 to 1.00)
	Male	35/72 (49)	83/132 (63)	0.77 (0.59 to 1.01)
Subcutaneous nodule	Female	0/46 (0)	45/71 (63)	0.02 (0.00 to 0.27)
	Male	0/72 (0)	32/132 (24)	0.03 (0.00 to 0.46)
Erythema	Female	3/46 (7)	45/71 (63)	0.10 (0.03 to 0.31)
	Male	4/72 (6)	29/132 (22)	0.25 (0.09 to 0.69)
Warmth	Female	3/46 (7)	25/71 (35)	0.19 (0.06 to 0.58)
	Male	3/72 (4)	8/132 (6)	0.69 (0.19 to 2.51)
Induration	Female	1/46 (2)	27/71 (38)	0.06 (0.01 to 0.41)
	Male	1/72 (1)	4/132 (3)	0.46 (0.05 to 4.02)

(Continued)

Pruritus	Female	3/46 (7)	21/71 (30)	0.22 (0.07 to 0.70)
	Male	2/72 (3)	8/132 (6)	0.46 (0.10 to 2.10)
Arm motion limitation	Female	8/46 (17)	7/71 (10)	1.76 (0.69 to 4.53)
	Male	4/72 (3)	11/132 (8)	0.67 (0.22 to 2.02)
Oedema	Female	1/46 (2)	7/71 (10)	0.22 (0.03 to 1.73)
	Male	0/72 (0)	3/132 (2)	0.31 (0.02 to 6.02)

Data source: [Pittman 2002](#).

AVA: adsorbed anthrax vaccine; CI: confidence interval.

^aData measured after 1 to 3 doses of AVA. We combined data by administration route: intramuscular and subcutaneous.

^bDenominator is number of doses administered.

Appendix 8. AVA (at weeks 0, 2, and 4): local reactogenicity^a

Reaction	Gender	Incidence (%) ^b		Risk ratio (95% CI)
		AVA intramuscular	AVA subcutaneous	
Warmth	Female	34/333 (10.2)	156/314 (49.7)	0.21 (0.15 to 0.29)
	Male	11/300 (3.7)	50/322 (15.5)	0.24 (0.13 to 0.44)
Tender-ness	Female	180/340 (52.9)	220/315 (69.8)	0.76 (0.67 to 0.86)
	Male	128/304 (42.1)	168/321 (52.3)	0.80 (0.68 to 0.95)
Itching	Female	17/332 (5.1)	71/312 (22.8)	0.23 (0.14 to 0.37)
	Male	7/300 (2.3)	22/319 (6.9)	0.34 (0.15 to 0.78)
Pain	Female	79/334 (23.7)	67/312 (21.5)	1.10 (0.83 to 1.47)
	Male	38/301 (12.6)	36/319 (11.3)	1.12 (0.73 to 1.72)
Arm motion limitation	Female	44/335 (13.1)	44/312 (14.1)	0.93 (0.63 to 1.37)
	Male	19/301 (6.3)	20/317 (6.3)	1.00 (0.54 to 1.84)
Erythema	Female	96/335 (28.7)	234/317 (73.8)	0.39 (0.32 to 0.47)
	Male	41/301 (13.6)	135/322 (41.9)	0.32 (0.24 to 0.44)
Induration	Female	33/332 (9.9)	134/312 (42.9)	0.23 (0.16 to 0.33)
	Male	18/300 (6.0)	72/321 (22.4)	0.27 (0.16 to 0.44)
Oedema	Female	50/334 (15.0)	86/312 (27.6)	0.54 (0.40 to 0.74)

(Continued)

	Male	21/300 (7.0)	69/320 (21.6)	0.32 (0.20 to 0.52)
Nodules	Female	21/332 (6.3)	161/313 (51.4)	0.12 (0.08 to 0.19)
	Male	5/300 (1.7)	72/319 (22.6)	0.07 (0.03 to 0.18)
Bruise	Female	15/333 (4.5)	18/310 (5.8)	0.78 (0.40 to 1.51)
	Male	12/300 (4.0)	11/317 (3.5)	1.15 (0.52 to 2.57)

Data source: [Marano 2008](#).

AVA: adsorbed anthrax vaccine; CI: confidence interval.

^aDuring clinic examinations.

^bThe denominator is the number of doses administered.

Appendix 9. rPA102 (different schedules) vs AVA: immunogenicity (anti-protective antigen antibodies)

Vaccine	Schedule	Geometric mean concentration ^{a,b}		Geometric mean titre ^{a,c}	
		µg/mL (95% CI)	Geometric mean ratio (95% CI)	95% CI	Geometric mean ratio (95% CI)
AVA	—	172 (109 to 269)	—	855 (553 to 1320)	—
rPA102	5 µg dose	4 (CI not in publication)	0.02 (CI cannot be calculated)	39 (CI not in publication)	0.05 (CI cannot be calculated)
	25 µg dose	12 (CI not in publication)	0.07 (CI cannot be calculated)	75 (CI not in publication)	0.09 (CI cannot be calculated)
	50 µg dose	26 (11 to 58)	0.15 (0.06 to 0.39)	374 (208 to 671)	0.44 (0.21 to 0.91)
	75 µg dose	44 (23 to 83)	0.26 (0.12 to 0.56)	515 (291 to 911)	0.60 (0.29 to 1.23)

Data source: [Gorse 2006](#).

AVA: adsorbed anthrax vaccine; CI: confidence interval; rPA102: recombinant protective antigen 102.

^aAt week 6 (after 2 doses of either vaccine). Geometric means to the base 10.

^bAnti-protective antigen.

^cToxic neutralization assay (TNA).

Appendix 10. rPA102 (different schedules) vs AVA: local reactogenicity

Vaccine dose	Gender	n/N (%)		Risk ratio (95% CI)
		AVA	rPA102 ^a	
1	Female	6/6 (100)	16/35 (46)	2.19 (1.52 to 3.14)

(Continued)

	Male	12/13 (92)	20/46 (43)	2.12 (1.47 to 3.06)
	Both	18/19 (95)	36/81 (44)	2.13 (1.63 to 2.78)
2	Female	5/6 (83)	17/35 (49)	1.72 (1.05 to 2.81)
	Male	11/13 (92)	11/44 (25)	3.38 (1.93 to 5.94)
	Both	16/19 (84)	28/79 (35)	2.38 (1.66 to 3.39)
3	Female	—	16/34 (47)	—
	Male	—	18/44 (41)	—
	Both	—	34/78 (44)	—

Data source: [Gorse 2006](#).

AVA: adsorbed anthrax vaccine; CI: confidence interval; n/N: number of participants with reactions/number of participants in treatment group; rPA102: recombinant protective antigen 102.

^aAll dose groups.

Appendix 11. rPA102 (different schedules) vs AVA: systemic reactogenicity

Vaccine dose	Gender	n/N (%)		Risk ratio (95% CI)
		AVA	rPA102 ^a	
1	Female	1/6 (17)	22/35 (63)	0.27 (0.04 to 1.62)
	Male	2/13 (15)	18/46 (39)	0.39 (0.10 to 1.48)
	Both	3/19 (16)	40/81 (49)	0.32 (0.11 to 0.92)
2	Female	2/6 (33)	16/35 (46)	0.73 (0.22 to 2.39)
	Male	2/13 (15)	7/44 (16)	0.97 (0.23 to 4.10)
	Both	4/19 (21)	23/79 (29)	0.72 (0.28 to 1.84)
3	Female	—	9/34 (26)	—
	Male	—	9/44 (20)	—
	Both	—	18/78 (23)	—

Data source: [Gorse 2006](#).

AVA: adsorbed anthrax vaccine; CI: confidence interval; n/N: number of participants with reactions/number of participants in treatment group; rPA: recombinant protective antigen 102.

^aAll dose groups.

Appendix 12. rPA102 vs AVA (intramuscular or subcutaneous): immunogenicity^a

Vaccine	Schedule	Geometric mean concentration ^{b,c}		Geometric mean titre ^{d,c}	
		µg/mL (95% CI)	Geometric mean ratio (95% CI)	95% CI	Geometric mean ratio (95% CI)
AVA	Intramuscular	200.4 (117.1 to 343.0)	—	1176.2 (694.1 to 1993.2)	—
	Subcutaneous	107.6 (52.8 to 218.9)	0.54 (0.22 to 1.31)	758.3 (493.0 to 1166.5)	0.64 (0.33 to 1.27)
rPA102	5 µg dose + Alhydrogel adjuvant	72.3 (25.7 to 203.4)	0.36 (0.11 to 1.16)	715.8 (317.2 to 1615.5)	0.61 (0.23 to 1.61)
	5 µg dose – Alhydrogel adjuvant	4.3 (1.3 to 15.6)	0.02 (0.01 to 0.08)	30.1 (3.1 to 292.8)	0.03 (0.00 to 0.26)
	25 µg dose + Alhydrogel adjuvant	59.6 (21.3 to 166.5)	0.30 (0.09 to 0.95)	641.2 (269.3 to 1526.9)	0.55 (0.20 to 1.50)
	25 µg dose – Alhydrogel adjuvant	10.0 (2.0 to 49.1)	0.05 (0.01 to 0.27)	79.2 910.8 to 583.5)	0.07 (0.04 to 0.12)
	50 µg dose + Alhydrogel adjuvant	171.7 (77.8 to 378.9)	0.86 (0.33 to 2.23)	1046.3 (410.2 to 2668.4)	0.89 (0.30 to 2.61)
	75 µg dose + Alhydrogel adjuvant	76.6 (16.1 to 364.7)	0.38 (0.07 to 1.99)	724.6 (233.9 to 2244.5)	0.62 (0.18 to 2.15)

Data source: [Campbell 2007](#).

AVA: adsorbed anthrax vaccine; CI: confidence interval; rPA102: recombinant protective antigen 102.

^aData measured at day 42. Enzyme-linked immunosorbent assay (ELISA) values, and lower confidence intervals that fell below the lower limit of assay quantification (2.5 µg/mL), were assigned an imputed value of 1.3 µg/mL and negative toxin neutralization activity (TNA) responses were assigned an imputed value of one.

^bAnti-protective antigen.

^cGeometric means to the base 10.

^dToxin neutralization.

Appendix 13. rPA102 vs AVA (intramuscular or subcutaneous): local adverse reactions

Vaccine	Schedule	Tenderness	Pain

(Continued)

		Incidence ^a (%)	Risk ratio (95% CI)	Incidence ^a (%)	Risk ratio (95% CI)
AVA	Intramuscular	10/20 (50)	—	14/20 (73)	—
	Subcutaneous	19/28 (68)	1.36 (0.82 to 2.25)	21/28 (75)	1.07 (0.75 to 1.53)
rPA102	5 µg dose + Alhydrogel adjuvant	7/19 (37)	0.74 (0.35 to 1.54)	13/19 (68)	0.98 (0.64 to 1.49)
	5 µg dose – Alhydrogel adjuvant	0/19 (5)	0.11 (0.01 to 0.75)	1/19 (0)	0.06 (0.00 to 0.99)
	25 µg dose + Alhydrogel adjuvant	8/20 (40)	0.80 (0.40 to 1.60)	13/20 (65)	0.93 (0.60 to 1.43)
	25 µg dose – Alhydrogel adjuvant	2/20 (10)	0.20 (0.05 to 0.80)	3/20 (15)	0.21 (0.07 to 0.63)
	50 µg dose + Alhydrogel adjuvant	10/20 (50)	1.00 (0.54 to 1.86)	13/20 (65)	0.93 (0.60 to 1.43)
	75 µg dose + Alhydrogel adjuvant	8/18 (44)	0.89 (0.45 to 1.75)	12/18 (67)	0.95 (0.62 to 1.47)

Data source: [Campbell 2007](#).

AVA: adsorbed anthrax vaccine; CI: confidence interval; rPA102: recombinant protective antigen 102.

^aDemonimator is number of injections, not number of participants.

Appendix 14. rPA102 vs AVA (intramuscular or subcutaneous): systemic adverse reactions

Vaccine	Schedule	Anorexia		Fatigue	
		Incidence ^a (%)	Risk ratio (95% CI)	Incidence ^a (%)	Risk ratio (95% CI)
AVA	Intramuscular	0/20 (0)	—	4/20 (20)	—
	Subcutaneous	3/28 (11)	4.50 (0.24 to 85.12)	5/28 (32)	0.89 (0.27 to 2.91)
rPA102	5 µg dose + Alhydrogel adjuvant	0/19 (0)	1.05 (0.02 to 50.43)	5/19 (26)	1.32 (0.41 to 4.18)
	5 µg dose – Alhydrogel adjuvant	1/19 (5)	2.21 (0.08 to 62.23)	3/19 (16)	0.79 (0.20 to 3.07)
	25 µg dose	0/20 (0)	1.00 (0.02 to 48.09)	2/20 (10)	0.50 (0.10 to 2.43)

(Continued)

+ Alhydrogel adjuvant

25 µg dose	2/20 (10)	4.20 (0.20 to 87.62)	4/20 (20)	1.00 (0.29 to 3.45)
------------	-----------	----------------------	-----------	---------------------

– Alhydrogel adjuvant

50 µg dose	1/20 (5)	2.10 (0.07 to 59.21)	5/20 (25)	1.25 (0.39 to 3.99)
------------	----------	----------------------	-----------	---------------------

+ Alhydrogel adjuvant

75 µg dose	2/18 (11)	4.67 (0.22 to 97.01)	4/18 (22)	1.11 (0.32 to 3.81)
------------	-----------	----------------------	-----------	---------------------

+ Alhydrogel adjuvant

Data source: [Campbell 2007](#).

AVA: adsorbed anthrax vaccine; CI: confidence interval; rPA102: recombinant protective antigen 102.

^aDemonimator is number of injections, not number of participants.

Appendix 15. rPA102 vs AVA (intramuscular or subcutaneous): unsolicited adverse events

Vaccine	Schedule	n/N (%)	Risk ratio (95% CI)	No. reactions
AVA	Intramuscular	5/10 (50)	—	13
	Subcutaneous	10/10 (100)	2.00 (1.08 to 3.72)	24
rPA102	5 µg dose	10/10 (100)	2.00 (1.08 to 3.72)	26
	+ Alhydrogel adjuvant			
	5 µg dose	7/10 (70)	1.40 (0.67 to 2.94)	22
	– Alhydrogel adjuvant			
	25 µg dose	5/10 (50)	1.00 (0.42 to 2.40)	23
	+ Alhydrogel adjuvant			
	25 µg dose	7/10 (70)	1.40 (0.67 to 2.94)	33
	– Alhydrogel adjuvant			
	50 µg dose	10/10 (100)	2.00 (1.08 to 3.72)	31
	+ Alhydrogel adjuvant			
	75 µg dose	4/10 (40)	0.80 (0.30 to 2.13)	8
	+ Alhydrogel adjuvant			

Data source: [Campbell 2007](#).

AVA: adsorbed anthrax vaccine; CI: confidence interval; rPA102: recombinant protective antigen 102; n/N: number participants with reactions/number of participants in the treatment group.

WHAT'S NEW

Date	Event	Description
23 January 2009	New citation required and conclusions have changed	<p>2009, Issue 2: A non-randomized trial that was included in the previous review version was excluded.</p> <p>We carried out a sensitivity analysis on a cluster-randomized trial included in the previous review version: the analysis showed the conclusions of the trial would change with only a small amount of clustering.</p> <p>New comparisons were carried out concerning doses and treatment routes.</p>
17 November 2008	New search has been performed	Search updated. New trials included in the review.

HISTORY

Protocol first published: Issue 2, 1997

Review first published: Issue 2, 2009

Date	Event	Description
15 November 2006	New search has been performed	2007, Issue 1 (new protocol published): The authorship has changed. The main changes are the addition of a secondary outcome measure 'specific antibody titre changes for anthrax' and the inclusion of trials with an active control group comparing dose regimens or administration routes.
14 May 2002	Amended	2002, Issue 3 (minor update): Revised 'Background', 'Description of studies', and 'Characteristics of included studies', and updated search.

CONTRIBUTIONS OF AUTHORS

This review replaces the [Jefferson 1998](#) Cochrane Review. All authors were involved in the adaptation of the protocol, with specific input from R Bellamy for the inclusion criteria, and S Donegan and C Gamble for the review methods. S Donegan and C Gamble assessed the trials for eligibility. S Donegan and R Bellamy carried out the data extraction. S Donegan carried out the analysis. All authors contributed to the writing of the final report.

DECLARATIONS OF INTEREST

None known.

SOURCES OF SUPPORT

Internal sources

- Liverpool School of Tropical Medicine, UK.

External sources

- Department for International Development (DFID), UK.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Deviations from the protocol published in 2007, Issue 1: We added 'seroconversion rate' as a secondary outcome measure. No meta-analysis could be undertaken therefore we could not explore heterogeneity, the robustness of the results to the influence of trial quality, or publication bias.

INDEX TERMS

Medical Subject Headings (MeSH)

Anthrax [*prevention & control]; Anthrax Vaccines [*therapeutic use]; Randomized Controlled Trials as Topic; Vaccines, Attenuated [therapeutic use]

MeSH check words

Humans