PHARMACOEPIDEMIOLOGY AND DRUG SAFETY 2007; **16**: 259–274
Published online 24 January 2007 in Wiley InterScience (www.interscience.wiley.com) **DOI**: 10.1002/pds.1359

ORIGINAL REPORT

Short-term reactogenicity and gender effect of anthrax vaccine: analysis of a 1967–1972 study and review of the 1955–2005 medical literature[‡,§]

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SUMMARY

Purpose In the 1960s, the Centers for Disease Control and Prevention (CDC) held the investigational new drug (IND) application for the anthrax vaccine and collected short-term safety data from approximately 16 000 doses administered to almost 7000 individuals. While some recent anthrax vaccine safety studies have suggested that women experience more injection site reactions (ISRs), to our knowledge the IND safety data were not previously examined for a gender-specific difference.

Methods We identified and analyzed a subset of the IND study data representing a total of 1749 persons who received 3592 doses from 1967 to 1972. Original data collection forms were located and information extracted, including: vaccine recipient's name, age at vaccination, gender, dose number, date of vaccination, lot number, grading of ISR, presence and type of systemic reactions. Overall and gender-specific rates for adverse reactions to anthrax vaccine were calculated and we performed a multivariable analysis.

Results We found an ISR was associated with 28% of anthrax vaccine doses; however, 87% of these were considered mild. Systemic reactions were uncommon (<1%) and most (70%) accompanied an ISR. Our dose-specific analysis by gender found women had at least twice the risk of having a vaccine reaction compared to men. Our age-adjusted relative risk for ISR in women compared to men was 2.78 (95%CI: 2.29, 3.38).

Conclusions Our results for both overall and gender-specific reactogenicity are consistent with other anthrax safety studies. To date, possible implications of these gender differences observed for anthrax and other vaccines are unknown and deserve further study.

KEY WORDS — *Bacillus anthracis*; anthrax vaccine absorbed (AVA); vaccine adverse effects; vaccine gender effects

Received 10 February 2006; Revised 5 October 2006; Accepted 24 October 2006

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[‡] The protocol for this study was approved by an Institutional Review Board of the Centers for Disease Control and Prevention.

[§] Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the funding agency.

INTRODUCTION

The Centers for Disease Control and Prevention (CDC) conducted the first human efficacy study of anthrax vaccine in the 1950s among textile mill workers.[1] At the time, workers who handled imported goat hair were at increased risk for anthrax disease in the United States.

During the 1960s, the CDC held the investigational new drug (IND) application for anthrax vaccine, and vaccine was sent to requesting physicians who agreed to collect short-term reactogenicity information on vaccinated individuals. In total, approximately 16 000 doses were administered to almost 7000 persons.[2,3] The CDC summarized this reactogenicity information in a series of five reports that covered the period 1966–1971.[2] These reports were submitted to support the safety of Anthrax Vaccine Adsorbed (AVA) and, together with the data from the efficacy study, led to licensure of AVA in 1970.

During the last decade, anthrax vaccine has been limited, almost exclusively, to use by the military. A gender difference where women experience more injection site reactions (ISRs) than men has been noted, initially in small vaccine trials conducted by the United States Army Medical Research Institute of Infectious Diseases and corroborated in other military studies.[4–6] This gender difference was an unexpected finding and the biologic significance of this finding is not understood. In addition, in the 1999 mandate that established the CDC's Anthrax Vaccine Safety and Efficacy Research Program, policy makers specifically directed the agency to conduct research studies to address this gender difference. This report re-examines and re-analyzes a subset of archival safety data collected as part of the CDC IND study conducted in the late 1960s and early 1970s. Anthrax vaccine reactogenicity, overall and gender-specific rates and a multivariable model were analyzed and implications of our results for future studies are discussed.

METHODS

Original study design of CDC IND 1960s study

Study design. The CDC IND study was a multi-site, prospective cohort study of persons at occupational risk for anthrax disease who were vaccinated.

Setting. During the CDC IND study, physicians who requested and received shipment of anthrax vaccine from CDC, and who completed vaccine safety forms were defined as 'investigators.' All current attempts to contact these original investigators have failed. A former nurse in her 80s was contacted from one of the larger vaccination sites and she was able to describe the method by which reactogenicity information was collected.

Required vaccination of all workers began on their date of hire and continued throughout their employment. Vaccinated workers consisted primarily of personnel in the textile industry in contact with goat hair or wool, research and diagnostic laboratory workers, and occupational health employees at nine U.S. and one foreign study site.[2] Any serious adverse reaction requiring medical care was brought to the attention of the medical staff administering the vaccine. Adverse reactions not requiring medical care were ascertained at the next scheduled vaccination. At that time, vaccine recipients were asked to describe any reactions that had occurred within 2 days of their last vaccination. No universal, objective evaluation of adverse reactions at 48 hours by medical staff was conducted. CDC data collection forms were completed for each vaccine recipient and periodically mailed back to CDC.

Vaccine. Two vaccine formulations were administered under the IND application. The first formulation of the anthrax vaccine was obtained by CDC from the U.S. Army, Fort Detrick, Maryland and was used in the 1950s efficacy study among textile mill workers.[1,7] It will be referred to as the Fort Detrick formulation in this report. It was manufactured by Merck, Sharp and Dohme and was only distributed during 1966, the first reporting year. The vaccine was distributed to physicians under an IND application. Each 0.5 ml dose of vaccine was to be administered subcutaneously according to the recommended vaccination schedule at 0, 2, 4 weeks, and 6, 12, 18 months and followed by yearly booster doses.

In the 1960s, this formulation was reformulated using a different anthrax strain, propagated under different growth conditions, with inclusion of an aluminum hydroxide adjuvant, benzalkonium chloride preservative, and formaldehyde as a stabilizer.[8] This reformulated vaccine represents today's AVA, and at the time was manufactured by the Michigan Department of Public Health.[8] AVA was renamed BioThrax™ in 2002 and has been manufactured by Bioport Corporation since December of 2001. It will be referred to as AVA in this report.

Because distribution of the first vaccine formulation was discontinued, some participants received both formulations over the course of their vaccinations.[9]

Retrospective analysis of the 1960s CDC IND study

Data source. A box of data collection forms from the CDC IND study was located among other archived CDC material. Subsequently, information was

DOI: 10.1002/pds

Table 1. Type of injection site reaction and accompanying definition

Type	Description
None	No injection site reaction
Mild	Erythema only; edema or induration which is measurable but 30 mm or less in any one diameter
Moderate	Edema or induration measuring greater than 30 mm and less than 120 mm in any one diameter
Severe	Any reaction measuring more than 120 mm in any one diameter or any reaction accompanied by marked limitation of motion of the arm or marked axillary node tenderness

extracted from data collection forms and included vaccine recipient's name, age at vaccination, gender, dose number, date of vaccination, lot number, grading of ISR, presence and type of systemic reaction, and investigator name. Miscellaneous remarks—such as 'lump,' 'red,' 'itchy,' 'painful,' or 'swollen'—which were added by the investigator were also extracted. This information was entered into EpiInfo [version 6.04; CDC, Stone Mountain, Georgia]. Vaccine recipient's full name, age, and investigator were used to link doses to an individual. Unique identifiers were then assigned and names deleted from the database. Multiple doses for the same person were linked and duplicate records deleted.

Definitions and coding. ISRs were graded according to Table 1. Systemic reactions were noted but not graded. For coding purposes, any injection site (i.e., mild, moderate, or severe) and/or systemic reaction that occurred following a dose of anthrax vaccine was considered positive for 'vaccine reaction.' If a written comment existed that the vaccine reaction was 'red,' 'swollen,' or 'slight' without quantifying measurements and the original grading of the ISR was either blank or indicated 'none,' then the reaction was coded or re-coded as 'mild.' Comments of pain, soreness, and lumps or nodules were considered as nonspecific and not used for re-coding purposes.

Gender, if missing, was assigned based on the recipient's first and/or middle name.

Missing lot numbers were assigned using a conservative algorithm based on the injection dates and lot numbers used by each investigator (Table 2). Lot information was completed for a dose if any one of the three algorithm conditions were satisfied. Overall, 3% (114/3337) of doses were determined using this algorithm. There were no substantial differences in prevalence by lot number with or without the algorithm; however, the algorithm was used to obtain more stable estimates.

A dose was considered complete if vaccination date and reactogenicity information were recorded. Numbers of complete doses were used as the denominator for reactogenicity per dose calculations. Total doses in this study were the sum of all doses with a vaccination date, with or without reactogenicity information. Total doses are only used in comparing the existing CDC IND data to what was originally reported in the IND reports, covering 1966–1971.

Dose-specific sub-analyses. Sub-analyses were restricted to those persons who received one or more of the first six doses of vaccine, the primary vaccine series. To ensure a more accurate estimate of reactogenicity, the timeliness of vaccination was assessed. Doses received according to the recommended vaccination schedule, plus or minus 20% of the recommended time interval were considered to be given 'on time.' Doses that did not meet this criterion were considered not on time and excluded from analysis. Individual doses or

Table 2. Unknown lot number assignment

Missing lot numbers were determined and assigned when any one of the three following conditions were met:

I. In circumstances where a specific MD only administered one particular vaccine lot for all known injections, injections with unknown lot numbers will be assigned the lot used in all other injections, providing that the injection with lot number to be assigned was not administered greater than 14 days from last known lot number injection.

II. In circumstances where a specific MD administered an injection with an unknown lot number on the same day as an injection with a known lot number, the unknown lot number will be assigned the known lot number of the injection given that same day. This is provided that the MD used no different known lot within 14 days of the injection where lot number is to be assigned.

III. In circumstances where a specific MD administered an injection with an unknown lot number within 7 days as an injection with a known lot number, the unknown lot number will be assigned the known lot number of the injection given with 7 days. This is provided that the MD used no different known lot within 21 days of the injection where lot number is to be assigned.

DOI: 10.1002/pds

nonsequential doses were, however, included. For example, if doses #4 and #6 were documented for a person, they were included. To avoid including persons with pre-existing immunity, only persons who were first time vaccine recipients or $\geq$10 years had elapsed since receipt of their last dose were included in the sub-analyses. Since there are limited data available on the durability of the immune response after anthrax vaccine, 10 years was selected as a reasonable time in which vaccine-induced immunity would wane.

Analytic methods. The Chi-square statistic was used to compare reactogenicity rates (overall and systemic) among men and women. The test incorporated a two-sided alternative and was conducted at a significance level of 0.05. Estimates of relative risk and 95% confidence intervals were calculated using Statistical Analysis Software (SAS) [version 9; SAS Institute, Cary, NC].

We also fitted a multivariable model and estimated risk ratios and 95% confidence intervals using the SAS PROC GENMOD procedure. We used only completed 'on time' vaccine doses #1–6, which included gender, age category, date of administration, and local reactogenicity information. To address repeated measurements on subjects, a Generalized Estimating Equations (GEE) approach was used in parameter estimation. Our outcome was occurrence of an ISR, and potential covariates we examined for inclusion in our model included gender, age (four categories: 18–24, 25–34, 35–44, and > or equal 45 years), lot number (2, 3, 7, 8, 9), and number of doses received.

RESULTS

From 28 September 1967 to 29 July 1972, a total of 1749 persons received 3592 doses of anthrax vaccine from 20 investigators. A total of 3337 doses were complete, including date of administration and reactogenicity information. Of these 3337 doses, 3331 included gender information. Examination of only the primary vaccine series' doses that were given 'on time,' limited the number of eligible doses to 1884; however, 1879 doses had gender information (Figure 1), and 1598 had both gender and age information.

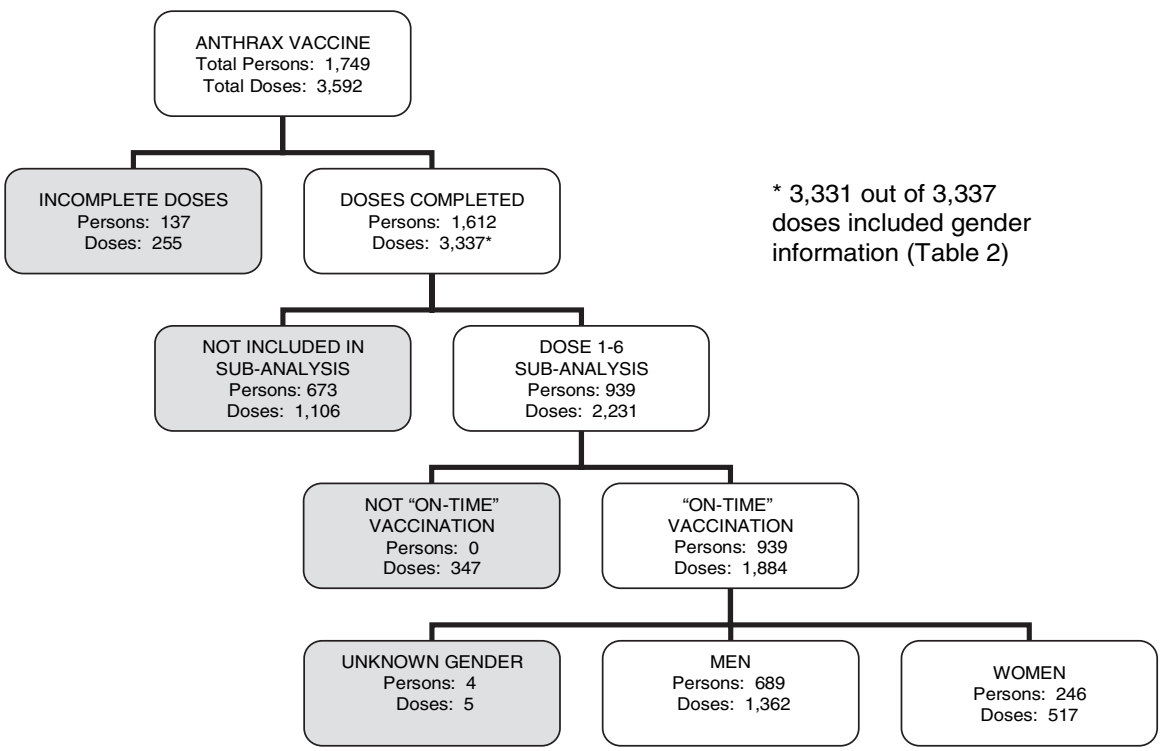

Figure 1. Distribution of anthrax vaccine, 1967–1972

DOI: 10.1002/pds

The primary vaccine series included six doses administered according to the vaccination schedule at 0, 2, 4 weeks and 6, 12, 18 months, followed by yearly boosters. Because required vaccination of all workers began on their date of hire and continued throughout their employment, our analysis included a cross-section of persons beginning the vaccination series with dose #1 to those receiving dose #34. The mean (and median) number of doses received per person was two, ranging from one to six doses received. Women comprised 27% of this study group, and those for whom gender was missing and could not be unambiguously assigned comprised <0.5% of persons. Study participants ranged in age from 16 to 71 years. The median age of study participants was 30 years, the 25th percentile and 75th percentile ages were 22 and 46 years, respectively; however, over one-third of study participants' ages were missing.

ISRs were associated with 28% (925/3337) of doses (Table 3). Mild ISRs defined as reactions with 'erythema only; edema or induration which is measurable but 30 mm or less in any one diameter' occurred in almost one-quarter (24%; 805/3337) of doses and accounted for 87% (805/925) of all reported ISRs. Moderate ISRs defined as reactions with 'edema or induration measuring greater than 30 mm and less than 120 mm in any one diameter' was reported in 3% (114/3337) of doses. Severe ISRs were defined as being 'any reaction measuring more than 120 mm in any one diameter' or 'any reaction accompanied by marked limitation of motion of the arm or marked axillary node tenderness' and occurred in <1% (6/3337) of doses. Of the severe ISR, two reports followed receipt of dose #2; the remaining four were after a booster dose.

Most systemic reactions (70%; 14/20) were accompanied by an ISR. Systemic reactions were uncommon, <1% (20/3337) of doses. When they did occur, the majority (65%; 13/20) of systemic reactions occurred during the primary vaccine series, doses #1–6, with the greatest number reported after receipt of dose #2 (25%; 5/20).

A total of 20 systemic reactions were reported by 19 individuals. One individual (a male) reported two systemic reactions to two different doses. Of the 20 systemic reactions, gender information was recorded for 19 individuals and 9 were women. Age was available for 18 of the 20 doses and approximately 72% (13/18) of those persons were aged ≥40 years. When the analysis was limited to only the primary vaccine series, 12 persons experienced systemic reactions to 13 doses. Of the 12 persons, age was available for 11 and approximately 64% (7/11) were aged ≥40 years; of these, 57% (4/7) were men.

Types of systemic reactions were recorded in all but two instances. Of the 18 reactions for which symptoms were listed, fever with and without chills was the most commonly cited symptom (56%; 10/18), followed by headache (28%; 5/18) and nausea (17%; 3/18).

Reactogenicity varied by vaccine lot (Table 3). Five vaccine lots were administered to study participants: Lots 2, 3, 7, 8, and 9. Lot 7 made up the majority (73%; 2203/3021) of doses with a known lot and Lot 9 represented the early Fort Detrick formulation and comprised (7%; 218/3021) of these doses.[2] Vaccine Lots 2, 3, and 7 are documented to be AVA.[5] Lot 8 was presumed to also be AVA, since it was first administered beginning in May, 1972, well after the initial use of AVA lots. Lot 6 was also administered, however, the formulation (Ft. Detrick or AVA) is not known. Three doses of Lot 6 were administered to one woman.

Table 3 includes lot-specific and overall reactogenicity by type of reaction for completed doses (3337). Lots 2 and 7 were the least reactogenic and Lots 3 and 8 the most reactogenic. Lot 9's reactogenicity profile was intermediate, falling between the extremes of AVA reactogenicity. For those doses where gender was reported (3331), Lots 2 and 7 were the least reactogenic and Lots 3 and 8 the most reactogenic. Lot 9's reactogenicity profile was intermediate, falling between the extremes of AVA reactogenicity. Reactogenicity profiles for men showed Lots 2, 7, and 9 to be the least reactogenic and Lots 3 and 8 to be the most reactogenic. Reactogenicity results for women by vaccine lot showed Lots 2 and 7 to be the least reactogenic, and Lots 3 and 8 were the most reactogenic, while Lot 9's reactogenicity profile was intermediate. Only two mild ISRs were reported for Lot 6.

Dose-specific vaccine reaction prevalence varied from 25% of doses after dose #1 to 35% of doses after dose #5 (Table 4). Furthermore, there was a statistically significant increase in vaccine reaction prevalence after dose #2 (32%) when compared with dose #1 (25%).

Dose-specific analysis by gender found women were at increased risk of having a vaccine reaction than men after doses, #1–6 (Table 4). At a minimum, women's risk for vaccine reactions was at least twice that of men.

Multivariable model

We fit a multivariable model using an exchangeable working correlation structure. First, a base model was constructed which included only the covariates gender and age category. Using a manual stepwise method,

DOI: 10.1002/pds

Table 3. Lot-specific, overall, and gender-specific reactogenicity of anthrax vaccine by type of reaction

LOT #	Gender	Doses	Injection site reaction (mild, moderate, and severe)		Mild injection site reaction		Moderate injection site reaction		Severe injection site reaction		Systemic reaction	
			Number	% (95%CI)	Number	% (95%CI)	Number	% (95%CI)	Number	% (95%CI)	Number	% (95%CI)
2	Men	192	33	17.2	21	11.0	11	5.7	1	0.5	2‡	1.0
	Women	93	37	39.8	26	28.0	11	11.8	0	0.0	2‡	2.2
	Overall	288*	71	24.7 (20.0– 29.9)	48*	16.7 (12.8–21.4)	22	7.6 (5.1–11.3)	1	0.3**	5*,‡	1.7**
3	Men	49	21	42.9	17	34.7	3	6.1	1	2.0	0	0.0
	Women	38	26	68.4	17	44.7	9	23.7	0	0.0	1‡	2.6
	Overall	87	47	54.0 (43.6–64.1)	34	39.1 (29.5–49.6)	12	13.8 (8.1–22.6)	1	1.1**	1	1.1**
7	Men	1590	217	13.6	197	12.4	19	1.2	1	0.1	7‡	0.4
	Women	610	330	54.1	291	47.7	37	6.1	2	0.3	5‡	0.8
	Overall	2203*	548	24.9 (22.1–26.7)	489*	22.2 (20.5–24.0)	56	2.5 (2.0–3.3)	3	0.1**	12	0.5**
8	Men	145	46	31.7	45	31.0	1	0.7	0	0.0	0	0.0
	Women	77	56	72.7	53	68.8	3	3.9	0	0.0	0	0.0
	Overall	222	102	45.9 (39.5–52.5)	98	44.1 (37.8–50.7)	4	1.8†	0	0	0	0
9	Men	145	25	17.2	23	15.9	2	1.4	0	0.0	0	0.0
	Women	73	49	67.1	42	57.5	7	9.6	0	0.0	0	0.0
	Overall	218	74	33.9 (28.0–40.5)	65	29.8 (24.1–36.2)	9	4.1 (2.2–7.7)	0	0.0**	0	0.0
Missing	Overall	316	81		69		11		1		2	
Total	Overall	3337*,†	925	27.7 (26.2–29.2)	805*	24.1 (22.7–25.6)	114	3.4 (2.9–4.1)	6	0.2**	20*	0.6 (0.3–0.9)

*Number of doses shown is greater than total number of doses when gender specified since not all doses included gender information. Number of doses when gender specified = 3331/3337 (99.8%).

**Standard deviation ≥30% of the estimate; value unstable.

†Three doses from Lot 6 (not shown in table) are included in overall total (3337). Lot 6 was administered to only one woman and two mild injection site reactions were reported. Formulation (Ft. Detrick or AVA) for Lot 6 is unknown.

‡The following systemic reactions were accompanied by injection site reactions. Lot 2: Three of the systemic reactions in men, two of systemic reactions in women, and one systemic reaction where gender was not specified were accompanied by an injection site reaction. Lot 3: The systemic reaction experienced by the woman was accompanied by an injection site reaction. Lot 7: Three of the systemic reactions in men and three of the systemic reactions in women were accompanied by an injection site reaction. Lot 9: The systemic reaction experienced by the woman was accompanied by an injection site reaction.

DOI: 10.1002/pds

Table 4. Anthrax vaccine reactions, doses #1–6, overall and gender-specific*

	Overall				Gender-specific		
	Doses* ($n = 1879$)	Number	% (95%CI)		Doses* ($n = 1879$)	Number (%)	RR (95%CI)
Dose 1	705	173	25 (22–28)	Men	521	84 (16)	3.0 (2.3–3.8)
				Women	184	89 (48)	
Dose 2	453	146	32 (28–37)	Men	323	65 (20)	3.1 (2.4–4.0)
				Women	130	81 (62)	
Dose 3	334	104	31 (26–36)	Men	233	42 (18)	3.4 (2.5–4.6)
				Women	101	62 (61)	
Dose 4	111	28	25 (18–34)	Men	85	13 (15)	3.8 (2.1–6.9)
				Women	26	15 (58)	
Dose 5	124	44	35 (27–44)	Men	91	25 (27)	2.1 (1.3–3.3)
				Women	33	19 (58)	
Dose 6	152	39	26 (19–33)	Men	109	20 (18)	2.4 (1.4–4.0)
				Women	43	19 (44)	

*Number of doses shown only include doses in which gender information was specified = 1879/1884 (99.7%).

we evaluated adding separately to the model the covariates dose and lot number. Dose was not found to be statistically significant and dropped from the model. Although lot number was statistically significant, further pairwise comparisons of individual lot numbers found only the comparison of Lot 8 versus Lot 9 was statistically significant. In addition, the impact of adding lot number on the age-adjusted relative risk estimate of gender for ISR was negligible and thus we chose the base model as our final model. Furthermore, age category alone and the interaction term (gender × age category) were both not statistically significant. Our results showed the age-adjusted relative risk for ISR in women compared to men was 2.78 (95%CI: 2.29, 3.38).

DISCUSSION

This report examines a subset of archival safety data collected as part of the CDC IND study conducted in the 1960s. Safety data were collected on CDC data collection forms if the vaccine recipient reported any reactions that had occurred within the past 2 days of their last vaccination when asked by the medical staff. Although the recipients self-reported their adverse events at their next scheduled vaccination, it is unclear the extent to which the reaction data were solicited by investigators at the time the data were collected. Therefore, actual reports may be higher if investigators prompted participants to report every symptom. However, this information is unknown.

Since this report examines a subset of archival safety data collected as part of the CDC IND study, the data used in our re-analysis were not complete. Only a fraction (3592 of approximately 16 000 doses) of the original IND data were found. The period of observation for our study differed from that of the IND summary reports, 1967–1972 versus 1966–1971, respectively. A thorough search of CDC and CDC's archived material has, to date, been unsuccessful in locating additional data. It is believed that whatever other data might have existed were destroyed when the expiration/destruction date, usually 25 years, was reached.

Overall, our results for both overall and gender-specific reactogenicity analyzed based on the number of completed doses (3337) are consistent with other anthrax vaccine safety studies. Furthermore, our multivariable analysis found the age-adjusted relative risk for ISR in women compared to men was 2.78 (95%CI: 2.29, 3.38).

However, when comparing adverse reactions reported in our analysis to other studies, it is important to note that there are differences in the ways that each study was conducted. Differences in data collection methodology including, but not limited to, definitions of adverse events and severity of these events, periods post vaccination when adverse events data were collected, active or passive surveillance, and whether adverse events were reported based on number of participants or number of doses, all need to be considered.[9] Table 5 shows a comparison of injection site and systemic adverse events (reported by gender when data are available) for selected historical anthrax vaccine (AVA) safety studies. ISRs reported in the table (for subcutaneous administration) ranged from 0.1% to 93% overall and the ISRs for men ranged from 0.03% to 65% and for women 0.1% to 93%,

DOI: 10.1002/pds

Table 5. Anthrax Vaccine Safety Studies, 1955–2002*

Study	Period of observation	Formulation	Number of participants or doses	Study design	Overall rates of adverse events[a]
Studies of adverse events following anthrax vaccination					
Controlled trials					
The Brachman Study[1,9]	1955–1959	Ft. Detrick	1249 Participants	Randomized, placebo controlled trial among mill workers (four mills) who processed imported goat hair Active surveillance Participants examined at 24 and 48 hours (two mills) AEs reported based on number of participants	Injection site reactions[b] (35.0%) Systemic reactions[c] (0.2%)
United States Army Medical Research Institute of Infectious Diseases (USAMRIID) Reduced Dose, Route Change Study[5,9]	1998	AVA	173 Participants (109 men; 64 women)	Prospective, randomized, open label study to evaluate the standard schedule versus reduced dose, route comparison SQ to IM	Injection site reactions[d] Subcutaneous (men: 2.3–62.9%; women: 9.9–84.5%) Intramuscular (men: 0.0–48.6%; women: 0.0–67.4%) Systemic reactions[e]
				Active surveillance Participants examined at 30 minutes, 1–3 days, 1 week, and 1 month after each vaccination AEs reported based on number of doses	Subcutaneous (men: 2.3–9.8%; women: 0.0–11.3%) Intramuscular (men: 0.0–6.9%; women: 0.0–17.4%)
Uncontrolled trials					
CDC IND Reports[2,9]	1966–1971	Ft. Detrick and AVA	6895 Participants ≈16 000 doses	Observational; assessed use in high-risk settings Unclear if passive or active surveillance[i] Participants asked to report any AEs experienced 48 hours post vaccination at next scheduled vaccination AEs reported based on number of doses and participants	Injection site reactions[f] (0.2–8.4%) Systemic reactions[g] (0.06%)
CDC IND Reports Sub-study	1967–1972	Ft. Detrick and AVA	1749 Participants, 3337 Doses	Observational; assessed use in high-risk settings Unclear if passive or active surveillance[h]	Injection site reactions[i] (28%) Systemic reactions (0.6%)

DOI: 10.1002/pds

				Participants asked to report any AEs experienced 48 hours post vaccination at next scheduled vaccination AEs reported based on number of doses	
Ft. Bragg Booster Study[9,35]	1992 and 1994	AVA	495 Participants 452 Participants received both AVA and pentavalent botulinum toxoid (PBT) 43 Participants received AVA only	Assessment of safety of booster doses given to soldiers Active surveillance Participants assessed at 30 minutes, 1, 2, 3, 7 days, and once between 24 and 36 days post vaccination AEs reported based on number of participants	Injection site reactions[j] (4.7–27.9%) Systemic reactions[k] (2.5–23.3%)
Canadian Forces Safety Survey[34,36,l]	February 1998–April 1998	AVA	576 Participants 1676 Doses (1, 2, or 3 doses per person)	Cohort of Canadian soldiers deployed to Persian Gulf Active surveillance AEs reported based on number of participants	Injection site reactions[m] (0.5–12.7%) Systemic reactions[n] (5.7%)
Tripler Army Medical Center[9,34,37,38]	1998–2000	AVA	601 Participants (416 men; 185 women)	Prospective, self-report survey of medical support personnel Passive surveillance AEs reported based on number of participants	Injection site reactions[o] (men: 7–65%; women: 8–93%) Systemic reactions[p] (men: 60–67%; women: 62–80%)
U.S. Forces Korea[34,39]	1998–1999	AVA	2824 Participants (2214 men; 610 women)	Systematic recording of self-reported surveys after vaccine Passive surveillance AEs reported based on number of participants	Injection site reactions[q] (men: 0.4–29%; women: 2–62%) Systemic reactions[r] (men: 1–8%; women: 3–37%)
USAMRIID Short-Term Safety Study[4]	1973–1999	AVA	1583 Participants, 10 722 doses (men: 1249, 8797 doses; women: 334, 1925 doses)	Short-term, self-reported adverse events temporally associated with AVA for more than 25 years of use for at-risk personnel Passive surveillance AEs reported based on number of doses	Injection site reactions[s] (3.6%) (men: 0.03–2%; women: 0.1–6.4%) Systemic reactions[t] (1%) (men: 0–0.3%; women: 0.2–0.7%)

(*Continues*)

DOI: 10.1002/pds

Table 5. (Continued)

Study	Period of observation	Formulation	Number of participants or doses	Study design	Overall rates of adverse events[a]
CDC's Anthrax Vaccine and Antimicrobial Availability Program[40]	2001–2002	AVA	1727 Participants received 40 additional days of antimicrobial prophylaxis (i.e., ciprofloxacin, doxycycline, and amoxicillin)	Observational; Assessed persons who had potential significant exposure to *B. anthracis* and were recommended to take 60 days of antimicrobial prophylaxis after the anthrax attacks in the fall of 2001	Injection site reactions[u] (18.6–70.9%)
			199/1727 Participants received 40 additional days of antimicrobial prophylaxis and 3 doses of AVA	Participant diaries and 2-month telephone follow-ups were compared for those receiving antibiotic only and those receiving both antibiotics and AVA	Systemic reactions[v] (1.5–33.7%)
			1528/1727 Participants received 40 additional days of anti microbial prophylaxis only (without AVA)	Passive surveillance (diaries) and active surveillance (telephone follow-ups) AEs reported based on number of participants	
VAERS Report Studies					
Review by the Anthrax Vaccine Expert Committee (AVEC) of AEs reported to the Vaccine Adverse Event Reporting System (VAERS)[41]	1998–1999	AVA	602 VAERS reports reviewed	The AVEC, a civilian panel of private-sector physicians and other scientists reviewed VAERS reports using the Delphic approach (structured expert consensus) to assess the causal relationship between vaccination and the reported AEs and sought to identify unexpected patterns in occurrence of medically important events	Injection site reactions[w] (2.0–18.4%)
				AEs reported based on number of VAERS reports	Systemic reactions[x] (1.0–20.8%)
Expanded review of initial report on safety of AVA (see study above) by AVEC of AEs reported to VAERS[42]	1998–2001	AVA	1841 VAERS reports reviewed	The AVEC used the Delphic approach to tentatively assess causality to detect serious and medically important AEs	Injection site reactions[y] (1.7–15.9%)
				AEs reported based on number of VAERS reports	Systemic reactions[z] (1.0–13.8%)

*Dates correspond to period of observation of study participants.
[a]Overall rates of adverse events reported by gender when data are available.
[b]Two measures of injection site reactions were used: (1) 'erythema value,' based on the size of the area of erythema at the injection site and (2) a 'reaction index,' based on all findings of erythema, induration, and edema.
[c]Systemic reactions were not seen except for 2 individuals (2/1249) who experienced, along with the edema-producing location reactions, some malaise of 24 hours duration.

[d]Injection site reactions reported after 1–3 doses of AVA (% by doses). Total number of first three doses administered: IM = 118; SQ = 203. Range of reactions reported; reactions include tenderness, SQ nodule, erythema, warmth, induration, pruritus, arm motion limitation, and edema.
[e]Systemic reactions reported after 1–3 doses of AVA (% by doses). Total number of first three doses administered: IM = 118; SQ = 203. Range of reactions reported; reactions include headache, anorexia, malaise, myalgia, nausea, respiratory difficulty, general pruritus, and fever.
[f]Injection site reactions were reported based on number of doses and rated mild to severe. Range of reactions reported; mild reactions were defined as erythema only or measurable edema or induration of ≤3 cm in diameter, moderate reactions were defined as edema or induration of >3–12 cm in diameter, and severe reactions included any reaction >12 cm.
[g]Systemic reactions reported in 4 of 6895 participants. These reactions included chills, fever, aches, and malaise.
[h]It is unclear whether investigators relied on participants to report AEs (passive surveillance) or if they contacted participants to ascertain reactions (active surveillance).
[i]Injection site reactions rated mild to severe. Mfild reactions were defined as erythema only or measurable edema or induration of ≤3 cm in diameter, moderate reactions were defined as edema or induration of >3–12 cm in diameter, and severe reactions included any reaction >12 cm.
[j]Injection site reactions for participants who received an *AVA booster only* are reported and were defined according to Erythema/Induration index (E/I). Range of reactions reported; reactions ranged from values of E/I reaction of <5 cm in diameter and E/I reaction of 5–12 cm.
[k]Range of systemic reactions reported included for those receiving an *AVA booster only*. Systemic reactions included muscle ache, headache, malaise, joint pain, and fever.
[l]Reference 36 refers to a related study in a similar cohort of Canadian military forces vaccinated prior to deployment which found no evidence anthrax vaccination resulted in increased adverse health effects in a 8-month period after return from deployment.
[m]Injection site reactions were rated mild to severe. Range of reactions reported; mild reactions were considered to be 1–5 cm and were reported after 4.4% doses. Moderate reactions were considered to be >5–12 cm and reported after 0.2% doses. No large or severe reactions were reported.
[n]Systemic reactions occurred after 2.2% doses. Reactions included headache, flu-like gastrointestinal symptoms, fever with or without chills, foul taste in mouth, and neurology symptom.
[o]Range of injection site reactions reported and included redness with diameter of >5 cm, lump or knot at injection site, and edema.
[p]Range of systemic reactions reported and only included rates for muscle soreness.
[q]Range of injection site reactions reported and included rates for nodules, mild (redness < 5cm), moderate (redness 5–12 cm), and large (redness > 12 cm) reactions.
[r]Range of systemic reactions reported included itching, fever, chills, and malaise.
[s]One or more injection site reactions were reported in 3.6% of doses of AVA. Range of reactions reported included induration, erythema, tenderness, warmth, pruritis site, lymph node, arm motion limitation, edema, and rash.
[t]Systemic reactions reported in 1% of the doses in primary vaccination series included headache, malaise, myalgia, fever, nausea, vomiting, dizziness, chills, diarrhea, hives, anorexia, arthralgias, diaphoresis, blurred vision, generalized itching, and sore throat. Range of reactions reported by gender and only included headache, dizziness, and hives.
[u]Range of injection site reactions in those receiving both antibiotics and 3 doses of AVA reported on diaries included: bruise, warmth, soreness, redness, lump or 'knot,' arm motion limitation, itching on arm, and swelling.
[v]Range of systemic reactions in those receiving both antibiotics and 3 doses of AVA reported on diaries included; loss of appetite, diarrhea or stomach pain, heartburn, shortness of breath, swelling of lips/tongue or face, fatigue, headache, joint swelling or pain, muscle aches, nausea and/or vomiting, pain with swallowing, itching (other than arm), seizures, fever, rash or hives, chills, fainting, and vaginal yeast infection.
[w]Range of injection site reactions reported included: SC nodule, inflammation (<3 cm, 3–5 cm, >5–12 cm, >12 cm, and size unspecified), rash, and other event(s) at injection site.
[x]Range of systemic reactions reported included: reactions associated with the body as a whole (flu-like symptoms, malaise/fatigue, pain, not otherwise specified, fever, diaphoresis, syncope, chills, and weakness), digestive system (nausea, diarrhea, vomiting, oral symptom, and other gastrointestinal symptoms), respiratory system (dyspnea, throat symptom, and other respiratory symptoms), nervous system (headache, dizziness, paresthesia, memory loss, sleep disorder, altered mentation, other neurologic symptoms), integumentary system (rash, skin other, and pruritis), musculoskeletal system (arthralgia, myalgia, chest tightness, and chest pain), special senses (tinnitus and eye symptoms), urogenital system (any genitourinary symptoms), and cardiovascular system (heart rate/rhythm abnormality).
[y]Range of injection site reactions reported included: SC nodule, inflammation (<3 cm, 3–5 cm, >5–12 cm, >12 cm, and size unspecified), numbness and tingling, rash, and other event(s) at injection site.
[z]Range of systemic reactions reported included: reactions associated with the body as a whole (flu-like symptoms, malaise/fatigue, fever, pain, not otherwise specified, diaphoresis, chills, weakness, and syncope), cardiovascular system (heart rate/rhythm abnormality), digestive system (nausea, diarrhea, vomiting, oral symptom, and other gastrointestinal symptoms), hematologic/lymphatic system (swollen lymph nodes), integumentary system (rash, pruritis, and other skin symptoms), musculoskeletal system (arthralgia, myalgia, chest tightness, chest pain, arthritis, and other musculoskeletal symptoms), nervous system (headache, paresthesia, dizziness, altered mentation, memory loss, sleep disorder, and other neurologic symptoms), respiratory system (dyspnea, throat symptom, and other respiratory symptoms), and special senses (tinnitus and eye symptoms).

DOI: 10.1002/pds

respectively. Systemic reactions reported in the table (for subcutaneous administration) ranged from 0.0% to 80% overall and the systemic reactions for men ranged from 0.0% to 67% and for women 0.0% to 80%, respectively. In our analysis, participants passively reported any adverse events experienced 48 hours post last vaccination at their next scheduled vaccination. We found, 28% of vaccine doses were associated with ISR and less than 1% of doses administered were associated with systemic reactions.

In contrast, Brachman *et al*.,[1] used active surveillance which included an objective evaluation of reactogenicity at 24 and 48 hours in two out of the four textile mills post vaccination and participants reported an overall ISR rate of 35%. Two measures of ISR were used: (1) an 'erythema value' based on the measured area of injection site erythema observed at the injection site, and (2) 'reaction index' based on objective findings including erythema, induration, and edema. ISRs were rated mild to severe. Mild ISRs were defined as being 1–2 cm in redness and were seen in 30% of the participants. Common mild ISRs included erythema, pruritus, and a small area of induration. Moderate ISRs included injection site inflammation greater than 5 cm in diameter and were observed in 4% of the participants while severe ISRs were defined as extensive swelling of the forearm in addition to injection site inflammation and were only seen in three participants.

A recent study by the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) assessed short-term safety experience in volunteers who passively self-reported (i.e., returned to clinic to report symptoms only if they considered the adverse events should be reported and recorded in their medical record or if they wished treatment), and one or more ISR(s) were observed in only 3.6% of the doses administered.[4]

In addition, a pilot study at USAMRIID to compare use of fewer doses administered intramuscularly or subcutaneously with the current schedule and route, clinically and actively evaluated volunteers at four separate time points (30 min, 1–3 days, 1 week and 1 month) after each vaccination and reported ISR following subcutaneous vaccine administration occurred in 62% women and in 22% men.[5]

In the USAMRIID assessment of short-term safety report, Pittman *et al*.[4] acknowledged that their 'study of passively reported adverse events undoubtedly underestimates the true incidence of reactions, especially less severe reactions, due to limitations of the data collection.' Since the data collection method for our analysis was more rigorous than this USAMRIID study,[4] but less so than either of the Brachman or dose reduction and route change USAMRIID study,[1,5] it is not surprising that the prevalence of associated ISRs we found was intermediate. Of importance in both our analysis and Pittman *et al*.'s study,[4] self-report of vaccine reactions after weeks or months is subject to recall bias: mild reactions that come and go without problem tend to be forgotten, whereas more serious or debilitating reactions will be remembered.

In addition, some of the prevalence disparity in these studies may be attributed to inherent reactogenicity difference of early versus late doses—early, closely scheduled doses may lead to more sensitization reactions than later, less frequent doses. The doses received by participants in our analysis were skewed toward early vaccine series doses (median receipt of two doses) and Pittman *et al*.'s[4] passive self-reported study included more long-term recipients (median receipt of six doses).

Earlier work by Wright in the 1950s reported 2.4% of the first three anthrax vaccine doses were accompanied by ISR >5 cm and erythema/induration >5 cm.[7] Using the same ISR criteria, Pittman *et al*.[4] reported 1.6% of all doses had a similar reaction. In our analysis, approximately 4% of all completed doses developed ISR >3 cm (combined definition of moderate and severe ISR) which is more consistent with these two investigators' findings and suggests a threshold reporting difference for small ISRs.

Systemic reactions to anthrax vaccine are uncommon. Approximately 1% of doses were associated with report of a systemic reaction in our study which is consistent with other published reports.[1,4,7] It was an unexpected finding that the majority of persons experiencing systemic reactions following the primary vaccine series, doses #1–6, were men, since women experienced a greater burden of vaccine reactions, overall. Furthermore, the disproportionate representation of those aged $\geq$40 years experiencing systemic reactions was also surprising and in conflict with the findings of Pittman *et al*.[4] Since at-risk workers (primarily textile mill workers) comprised the pool of vaccinated individuals in our analysis, one possible explanation is that age $\geq$40 years may be a marker for long-term employment. Those who worked for years in the textile industry may have developed active immunity prior to their vaccination. When vaccinated, the immune worker experienced a more serious vaccine reaction than those exposed to the antigen for the first time. In recognition of the untoward reactions that may follow vaccination, a history of

DOI: 10.1002/pds

anthrax disease had always been a contraindication to receipt of the vaccine.[1]

Lot-to-lot variation in reactogenicity was observed in this analysis and has been reported by other investigators.[4,7] Wright reported ISR rates that varied by lot of the early Fort Detrick vaccine formulation and Pittman reported variation in lot reactogenicity of AVA. Since these published anthrax vaccine reactogenicity studies have used different vaccine lots than the ones examined in our analysis; consequently, no direct comparison of lot-to-lot reactogenicity could be made.

Because this study included one of the early Fort Detrick vaccine lots and four AVA lots, the reactogenicity of the two formulations can be compared. A study by Puziss and Wright[8] reported that there were no notable differences in ISR between the early Fort Detrick vaccine formulation and AVA, but no data have ever been presented. Our analysis included Lot 9 which was the early Fort Detrick vaccine formulation. Its reactogenicity profile was within the range of the AVA lots, suggesting that the reformulation had little effect on reactogenicity. Furthermore, the comparable proportions of adverse reactions observed in our analysis and Brachman's study, which used exclusively the early Fort Detrick formulation, also suggest similar short-term injection site reactogenicity of the two vaccine formulations.

Dose-specific vaccine reactions increased from dose #1 to dose #2. A sensitization of persons to the vaccine antigen after dose #1 may account for this increased reactivity after dose #2, as suggested by Wright and Pittman. The prevalence of vaccine reactions between men and women both increased by approximately 20% from dose #1 to dose #2 and suggested no gender-specific sensitization following dose #1. In contrast, data from Pittman *et al*.[5] suggested a preferential sensitization of women.

A female predominance in reactogenicity to anthrax vaccine was observed in this study and others.[4–6,37–39] It has been noted and recently documented by the IOM that women are more likely than men to experience and report erythema, injection site tenderness, subcutaneous nodules, itching, and edema.[9] Of interest, a number of diseases, including thyroid cancer, coronary artery disease, tuberculosis, and autoimmune disorders such as systemic lupus erythematosus have a gender predominance. Unless a gender reactogenicity difference is very apparent, as was the case with anthrax vaccine, this difference would have been missed since vaccine trial data are rarely examined by gender. Only recently have vaccine safety studies begun to look at gender. A comprehensive review of the literature has identified a number of vaccines for which a gender differential for immunogenicity or reactogenicity exists.

Vaccine immunogenicity studies have observed differences between men and women. Women have a greater primary antibody response to a hepatitis B vaccine than men.[10,11] A similar result was found for women who received measles vaccine[12] and 23-valent pneumococcal vaccine.[13,14] In contrast, immune response after rubella re-vaccination[15] was initially faster and greater in boys, but girls' parameters were equivalent by 10 weeks. In a booster vaccination study using bivalent diphtheria-tetanus vaccine, a greater antibody response to diphtheria occurred in boys than girls, irrespective of the route of vaccine administration;[16] antibody response to tetanus was significantly greater in boys than girls when vaccine was administered intramuscularly and equivalent when administered subcutaneously.[16] Whether it can be generalized that primary immune responses are greater among women—and, conversely, that booster responses are generally greater among men—is not known.

Vaccine safety data from spontaneous reporting systems also suggest that a greater burden of vaccine reactions are experienced by women. The Vaccine Adverse Event Reporting System (VAERS), a federal database of reported vaccine adverse reactions, has shown there is a female predominance in reporting of adverse events for those aged $\geq$10 years.[17] In Australia, more women than men aged $\geq$16 years had a severe smallpox vaccine reaction that required treatment with immune globulin.[18] Because the number of women and men who were vaccinated is not known for either the VAERS or Australian data, it is not known with certainty whether more women than men experienced proportionately more vaccine reactions. Nonetheless, these data support that difference by gender in reactogenicity may be a more common finding than originally thought.

Vaccine safety data have demonstrated a female predominance of adverse reactions to many vaccines such as rubella,[19] acellular pertussis,[20] hepatitis A,[20] hepatitis B,[21] diphtheria/tetanus,[16,22] tetanus,[23] rabies,[24] influenza,[25–29] measles,[30,31] Japanese encephalitis,[32] anthrax,[4–6,37–39] and malaria.[33] The number of vaccines for which a gender reactogenicity difference exists clearly indicates that this phenomenon extends beyond anthrax vaccine and suggests that all vaccines should be examined for gender differences.

Re-analysis of the existing IND data has revealed some limitations. Adverse reactions to anthrax vaccine

were probably under-reported 30 years ago. A bias not to report adverse reactions most likely would have existed then, especially if receipt of the vaccine was a condition of one's employment. Severe and moderate reactions were to be reported directly to CDC but no log or record of these reports has been found at CDC. After filing a report to CDC, if the investigator failed to also record the reported reaction on an individual's data collection form, this would further contribute to an underestimation of vaccine reactogenicity in this study.

Although our data are a subset of the original safety data, we were able to identify more systemic reactions than originally reported in the IND summary report—20 cases in our analysis compared with 4 cases in the IND.[3] Much of this discrepancy can probably be accounted for by the greatly enhanced data handling ability we have with today's computers. Nevertheless, questions as to how representative our data are of the original safety data arise.

Our results are consistent with other published studies, both for overall and gender-specific reactogenicity. We believe that gender differences in vaccine reactogenicity may be a common finding and one should consider looking for it.

This study supports the earlier finding of military researchers that a gender reactogenicity differential exists for anthrax vaccine, with women experiencing a greater prevalence of ISRs than men. The majority of adverse reactions reported in this study were mild ISRs. While these reactions may be noticeable to vaccinees, the biologic significance of this finding is unknown. It may be that individuals who experience some type(s) of ISR to anthrax vaccine have a greater immune response and produce more antibodies than those who experience no ISR. For anthrax vaccine, this may mean greater protection against anthrax disease among women than men. Although Pittman *et al*. studied both the occurrence of adverse reactions and anti-PA IgG responses in their volunteers, these investigators did not report any connection between them in their subjects.[35]

As future studies of AVA are developed, the gender differential should be explored using uniform methods to collect safety data, standard adverse reaction definitions, and objective evaluation of adverse reactions by clinical staff. New generation anthrax vaccines, including the recombinant *Bacillus anthracis* protective antigen (rPA) vaccine, should also employ these same standards so that reactogenicity comparisons between old and new vaccines can be made. To increase efficiency, complementary studies, exploring gender-specific mechanisms of reactogenicity, should be conducted as part of safety and immune studies. Elucidating and understanding the mechanism by which gender differences lead to different health outcomes may have great implications for medicine and health.

KEY POINTS

- Our results for the occurrence of both vaccine ISR (28%) and systemic reactions (<1%) are consistent with other anthrax vaccine safety studies.
- A female predominance in reactogenicity to anthrax vaccine was observed in this study and other anthrax vaccine safety studies. Our dose-specific analysis by gender found women have at least twice the risk of having a vaccine reaction compared to men and our multivariable analysis found the age-adjusted relative risk for ISR in women compared to men was 2.78 (95%CI: 2.29, 3.38).
- Our review identified other vaccines which have demonstrated a female predominance in the occurrence of adverse reactions, indicating that this phenomenon extends beyond the anthrax vaccine and suggesting that safety profiles for all vaccines including the recombinant *Bacillus anthracis* protective antigen (rPA) anthrax vaccine currently in development should be examined for possible similar gender differences.
- Further studies will be needed to better understand the possible underlying mechanisms and identify potential, effective prevention strategies for clinically significant gender-specific adverse reactions which may adversely impact a vaccine's safety and/or acceptance.

ACKNOWLEDGEMENTS

The authors wish to thank John Becher, who had knowledge of this archived material, and Philip Brachman and Philip J. Smith who reviewed this manuscript.

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DOI: 10.1002/pds