

Dose-Response Models for Inhalation of *Bacillus anthracis* Spores: Interspecies Comparisons

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Because experiments with *Bacillus anthracis* are costly and dangerous, the scientific, public health, and engineering communities are served by thorough collation and analysis of experiments reported in the open literature. This study identifies available dose-response data from the open literature for inhalation exposure to *B. anthracis* and, via dose-response modeling, characterizes the response of nonhuman animal models to challenges. Two studies involving four data sets amenable to dose-response modeling were found in the literature: two data sets of response of guinea pigs to intranasal dosing with the Vollum and ATCC-6605 strains, one set of responses of rhesus monkeys to aerosol exposure to the Vollum strain, and one data set of guinea pig response to aerosol exposure to the Vollum strain. None of the data sets exhibited overdispersion and all but one were best fit by an exponential dose-response model. The beta-Poisson dose-response model provided the best fit to the remaining data set. As indicated in prior studies, the response to aerosol challenges is a strong function of aerosol diameter. For guinea pigs, the LD₅₀ increases with aerosol size for aerosols at and above 4.5 μm. For both rhesus monkeys and guinea pigs there is about a 15-fold increase in LD₅₀ when aerosol size is increased from 1 μm to 12 μm. Future experimental research and dose-response modeling should be performed to quantify differences in responses of subpopulations to *B. anthracis* and to generate data allowing development of interspecies correction factors.

KEY WORDS: Anthrax; *Bacillus anthracis*; bioterrorism; dose-response; inhalation exposure; microbial risk assessment

1. INTRODUCTION

Accurate dose-response models for biological agents that could be used in deliberate malicious releases are necessary for several purposes. First, they will help to better specify required detection limits for sensor systems that might be deployed. Second, they can better inform first responders in the event of an attack. Thirdly, they are required to develop scientifically based clean-up standards and release criteria following an attack. Finally, they can help to more realistically estimate potential consequences from different attack scenarios.

In this study, a published dose-response model⁽¹⁾ for *Bacillus anthracis* (the causative agent for anthrax) inhalation is refined using nonhuman dose-response data found in the open literature. In developing this refined model, the variation in response (at a given dose) with aerosol diameter and interspecies differences in response are also explored.

Frequently cited estimates of inhalation anthrax human LD₅₀ (dose at which 50% of the subjects die) are disparate, ranging from 4,100 spores⁽²⁾ to between 8,000 and 10,000 spores.⁽³⁾ The disparity in reported LD₅₀ arises from use of different animal models, different strains and isolates,⁽⁴⁾ and different techniques for administration of the aerosol (e.g., full-head vs. mask apparatuses).

Although important for providing general information on species susceptibility to *B. anthracis*, LD₅₀

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data alone are not sufficient for developing a risk estimate for anthrax. Because inhalation of *B. anthracis* has a high associated hazard and because there exists the potential for widespread distribution of *B. anthracis* spores, it is important that the response of humans to a low number of spores is known accurately.^(1,5) Despite the need for detailed data on response at low dose, relatively few studies have estimated low-dose response to inhalation anthrax. Based on extrapolation of data from two studies,^(2,6) Peters and Hartley⁽⁵⁾ estimate that the LD₁ for inhalation anthrax may be as low as 1–3 spores. Using several dose-response data sets on inhalation exposure of primates to *B. anthracis*, Haas⁽¹⁾ observed that published dose-response models produced orders-of-magnitude differences in predicted low-dose response and that low-dose inhalation exposure to *B. anthracis* results in nonzero risk.

Because exposure to bioterror agents is life-threatening to human populations, dose-response models for many of the potential bioterrorism agents must be developed or refined using information from experiments. Prior to use of nonhuman data, the applicability of nonhuman dose-response data sets must be assessed and, possibly, interspecies adjustment factors for use in extrapolation to human dose response must be developed. One means for assessing whether different species have the same dose-response relationship for a particular pathogen involves determining whether dose-response data for the species may be pooled.⁽⁷⁾

In this study, guinea pig dose-response data for inhalation of *B. anthracis* are used to refine a dose-response model that was developed using primate data.⁽¹⁾ As demonstrated by its use in attacks on the Hart Senate Office Building and federal postal facilities in 2001, *B. anthracis* is a credible threat as a bioterror agent. Clean-up of facilities contaminated with *B. anthracis* spores was hampered by uncertainty over the distribution of spores in the contaminated areas, the lack of a risk-based standard to which to decontaminate, operational difficulties associated with maintaining a desired disinfectant concentration, and optimal relative humidity.⁽⁸⁾ Epidemiological investigations of an anthrax outbreak in Sverdlovsk, USSR, and sampling performed after the Hart Senate Office Building attack of 2001 indicate that secondary aerosolization of *B. anthracis* spores was not significant in either case.⁽⁹⁾ The authors of that study acknowledge that there is uncertainty in the scientific community regarding the potential for secondary aerosolization and describe experiments performed in the Hart Senate Office Building, one site of the

2001 anthrax attacks, in which secondary aerosolization was demonstrated experimentally. If it did occur, secondary aerosolization could produce a wider, less predictable exposure to the spores, and further indicates the importance of confidence in low-dose response estimates.

2. DATA UTILIZED

An extensive literature search was conducted to locate publicly available dose-response data. To be useful in this study, data were required to conform to the following standards:

- inhalation of a bolus challenge was the exposure route;
- the dose (in number) of inhaled spores was provided;
- the specific response was explicitly stated; and
- information (aerosol size and strain) was provided on the *B. anthracis* used in the experiments.

Two studies^(10,11) were identified in which adequate data were available to develop dose-response models for inhalation of *B. anthracis* spores. Experiments conducted in the two studies are summarized in Table I. In both studies, the response end-point was death of the test animal. As described below, data were identified in which dose and response were determined at various aerosol diameters. In development of a refined dose-response curve, only data corresponding to aerosol sizes less than 5 μm (slightly larger than one spore) were used. As shown below, aerosols with a diameter less than 5 μm produce higher mortality rates (lower LD₅₀) in rhesus monkeys and guinea pigs. So using only data from experiments in which the smallest possible aerosol size was used is expected to yield a conservative estimate of hazard.

Druett *et al.*⁽¹¹⁾ exposed rhesus monkeys and guinea pigs to the Vollum strain of *B. anthracis* at aerosol sizes ranging from 1 μm to 12 μm . In prior work, the dose response from this study was reported.⁽¹⁾ Druett determined that for relatively short dosing times (less than 30 minutes) the time required for an animal model to inhale a given dose of *B. anthracis* spores had no significant effect on the response of the animal to the cumulative dose. The authors also found that the mortality observed among animal models was a strong function of aerosol diameter, with the ratio of the LD₅₀ at one spore aerosol size to LD₅₀ at 12 μm particle size equal to 15.6 for the guinea pigs and 14.2 for the rhesus monkeys. The authors

Table I. Data Used in the Current Study (Data Sets for Aerosol Size <5 μm)

Study	<i>B. anthracis</i> Strain	Test Animal	Dose (Inhaled Spores)	Number of Test Subjects	Positive Responses ³	Negative Responses
Altboum <i>et al.</i> ⁽¹²⁾	Vollum	Guinea pig	20,000,000	7	7	0
			2,000,000	8	7	1
			200,000	12	10	2
			20,000	12	6	6
			2,000	8	0	8
			200	4	0	4
Altboum <i>et al.</i> ⁽¹²⁾	ATCC_6605	Guinea pig	3,000,000	10	10	0
			300,000	10	8	2
			30,000	10	3	7
			3,000	10	0	10
			300	6	1	5
			30	6	0	6
Druett <i>et al.</i> ⁽¹¹⁾	Vollum	Rhesus monkey ¹	70,320	8	1	7
			77,040	8	4	4
			108,720	8	5	3
			137,520	8	6	2
			155,520	8	5	3
			160,800	8	3	5
			240,000	8	8	0
			300,000	8	7	1
			398,400	8	8	0
			Druett <i>et al.</i> ⁽¹¹⁾	Vollum	Guinea pig ² exposed to 1 μm particles	19,820
40,828	32	18				14
76,230	32	21				11
118,000	32	28				4

¹ Doses are based on data from the original work assuming a respiration rate of 2.4 L/min.

² Doses are based on data from the original work assuming a respiration rate of 0.118 L/min.

³ In all cases response was mortality.

concluded that, for rhesus monkeys and guinea pigs, infectivity falls sharply for particle sizes above 5 μm .

Altboum *et al.*⁽¹²⁾ evaluated the virulence of two strains of *B. anthracis* (Vollum and ATCC 6605) on guinea pigs in a study of seven antibiotic treatments. In the original study, the virulence tests were used to determine the extent of damage done to different organs within the guinea pig bodies. In this study the number of positive responses was used in development of dose-response relationships. As seen in Table I, the range of doses in the Altboum study is much wider than that of the Druett study. Altboum *et al.* used the intranasal exposure route in order to study respiratory infection with *B. anthracis*. The reason for selecting intranasal exposure rather than aerosol exposure is not provided. Guinea pigs were selected because they are susceptible to *B. anthracis* at low dose, because anthrax develops rapidly in the guinea pig, and because guinea pigs can be protected by a number of vaccine preparations.

More than 17 additional dose-response studies whose dose-response data could not be used in this

study were found in the literature. Data from these studies were not used because exposure route was not inhalation,^(13–19) because the study was a vaccination study or the infection process was altered in some way,^(20–23) because dose or exposure was not fully described in the study,^(2,6,24,25) because the response to different strains was the objective and graded doses were not administered,⁽²⁶⁾ or because responses did not include intermediate responses (between 0% and 100% mortality).^(22,27) One of the studies whose data were not used⁽²²⁾ contained dose-response data for rabbits exposed to aerosols of the Ames strain of *B. anthracis* at an aerosol size of 1.2 μm . In that study, the response of vaccinated and unvaccinated New Zealand white rabbits to aerosol challenge (Ames strain) was observed at doses of 133 and 84 times the LD₅₀ for white rabbits. Based on unpublished data, the authors determined the rabbit LD₅₀ to be 1.1×10^5 spores. Because 100% mortality was observed among the controls at all doses in the rabbit study, dose-response models could not be developed for that data set and the viability of pooling

those data with other data could not be assessed. Consequently, the rabbit data were not used in this study.

3. METHODS

Individual sets of the data found in the literature were fit with the one-parameter exponential model and two-parameter beta-Poisson and log-probit models. Dose-response model parameters were estimated using the method of maximum likelihood (MLE). The exponential and beta-Poisson models are the two most commonly used models for modeling human response to exposure to food-borne pathogens⁽²⁸⁾ and have been used in modeling human response to pathogens in drinking water⁽²⁹⁾ and to inhalation exposure to pathogens.^(30,31)

Use of the exponential and beta-Poisson models is widespread because they are simple and can be derived from basic biological considerations.⁽³²⁾ The two-parameter models are more flexible than the exponential model in fitting data and have a long history of use in dose-response modeling. Other models that have been proposed for human response to pathogen exposure include the log-normal, log-logistic, extreme value, Weibull-gamma, exponential-gamma, Weibull-exponential, and shifted Weibull models.^(28,33) These other models were not fit to data because there is, to date, no physiological and other justification for their selection and because, in prior microbial dose-response studies, the mechanistic two-parameter (beta-Poisson) model has accounted for as much uncertainty as more highly parameterized models.

The exponential dose-response model is given by

$$P(d) = 1 - e^{-kd}, \quad (1)$$

where $P(d)$ is the probability of death at dose d and k is the probability that a single organism will initiate the response. The beta-Poisson model is approximated by

$$P(d) = 1 - \left[1 + \left(\frac{d}{N_{50}} \right) \cdot \left(2^{1/\alpha} - 1 \right) \right]^{-\alpha}, \quad (2)$$

where N_{50} is the median dose to give the response and α is the exponential fitting parameter. The log probit model is given by

$$P(d) = \phi \left(\frac{1}{q_2} \cdot \ln \frac{d}{q_1} \right), \quad (3)$$

where q_2 is the probit slope, q_1 is the scale parameter, and ϕ denotes the normal cumulative distribution function.

Parameter estimations were performed using the “R” statistical computing language.⁽³⁴⁾ For the exponential and beta-Poisson MLEs, the BFGS algorithm was used for nonlinear minimization. The Nelder-Mead algorithm was used for minimization for the log probit model. Models were considered to exhibit goodness of fit if the model minimized deviance was less than the 95% confidence value for the χ^2 distribution at degrees of freedom equal to the number of doses tested -1 . Confidence intervals for best fit models were determined using bootstrap analyses with 5,000 bootstrap samples drawn from the dose-response data sets.

Additional dose-response models were fit to pooled data for combinations of each of the individual data sets listed in Table I. Pooling was deemed acceptable when the difference between the minimized deviance of the pooled data model and the sum of the deviances of the individual data set models was less than the 95% confidence value for the χ^2 distribution at degrees of freedom equal to the sum of the number of parameters used in fitting individual data sets minus the number of parameters used in fitting the pooled data set.

4. RESULTS

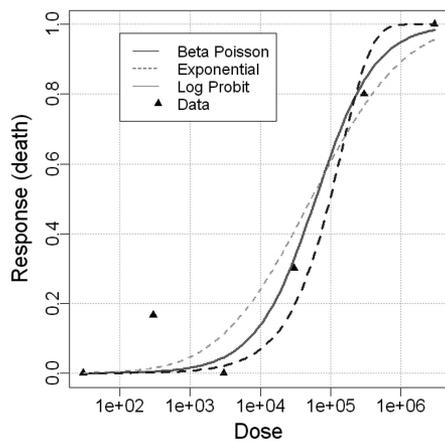
4.1. Dose-Response Model Development

Parameters and statistics for best fit models to all individual data sets and for a pooled data set of rhesus monkeys exposed to $1 \mu\text{m}$ aerosols of the Vollum strain pooled with guinea pigs exposed to the ATCC-6605 strain via intranasal instillation are presented in Table II. In Table II, GP denotes guinea pig and RM denotes rhesus monkey. Goodness of fit was realized for fits of all the individual data sets. Confidence intervals were determined via bootstrap analysis, as described above. In all cases where the two-parameter beta-Poisson dose-response model provided the better fit, the improvement in fit over the one-parameter model was demonstrated to be statistically significant. When fits for both two-parameter models were better than that of the one-parameter model, the best fit model was that which yielded the lowest deviance.

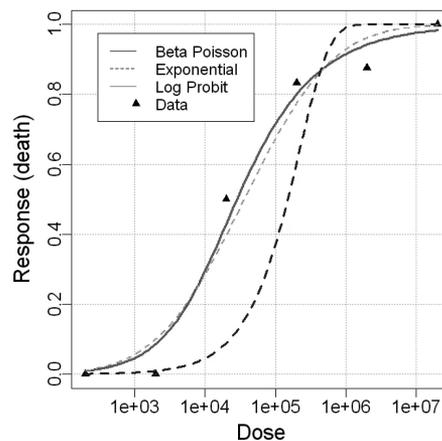
The beta-Poisson dose-response model provided the best fit for the data set for guinea pigs exposed to the ATCC-6605 strain of *B. anthracis*. The exponential model provided the best fit in all other cases. Plots showing fits of the three dose-response models (exponential, beta-Poisson, and log-probit) to individual data sets are shown in Fig. 1 and plots of the

Table II. Best Fit Models and Model Parameters

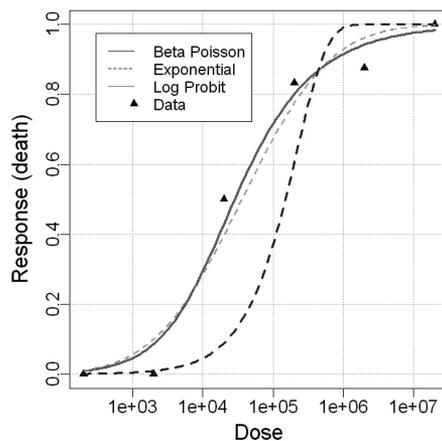
Data Set	Best-Fit Model	Parameter(s)	<i>P</i>	LD ₅₀ (Confidence Interval)	LD ₁₀ (Confidence Interval)
GP exposed to ATCC-6605 strain	Beta Poisson	$\alpha = 0.549$ $N_{50} = 28,472$	0.631	62,679 (20,760, 150,400)	7196 (826, 21,700)
GP exposed to Vollum strain	Exponential	$k = 7.51 \times 10^{-6}$	0.128	147,411 (17,400, 357,700)	23,095 (2730, 53,800)
Rhesus monkey data	Exponential	$k = 7.16 \times 10^{-6}$	0.188	92,000 (29,440, 70,932)	14,123 (10,787, 18,200)
GP, Vollum strain, 1 μm aerosol	Exponential	$k = 1.65 \times 10^{-5}$	0.699	41,930 (32,661, 53,346)	6360 (5005, 8212)
GP, ATCC strain pooled with RM	Exponential	$k = 7.15 \times 10^{-6}$	0.284	94,320 (74,100, 125,060)	14,800 (11,100, 19,600)



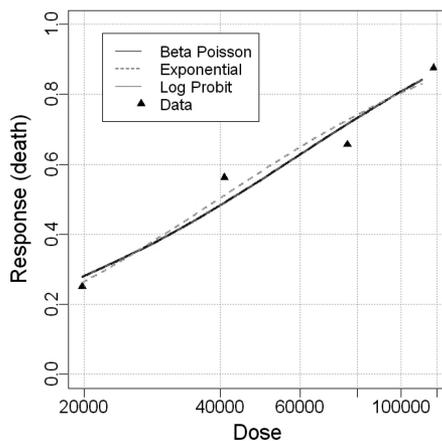
(a) Guinea pigs, ATCC-6605 Strain, i.n. exposure



(b) Guinea pigs, Vollum Strain, intranasal exposure



(c) Rhesus Monkeys, Vollum Strain, 1 μm aerosol



(d) Guinea pigs, Vollum Strain, μm aerosol

Fig. 1. Dose-response model fits to individual data sets.

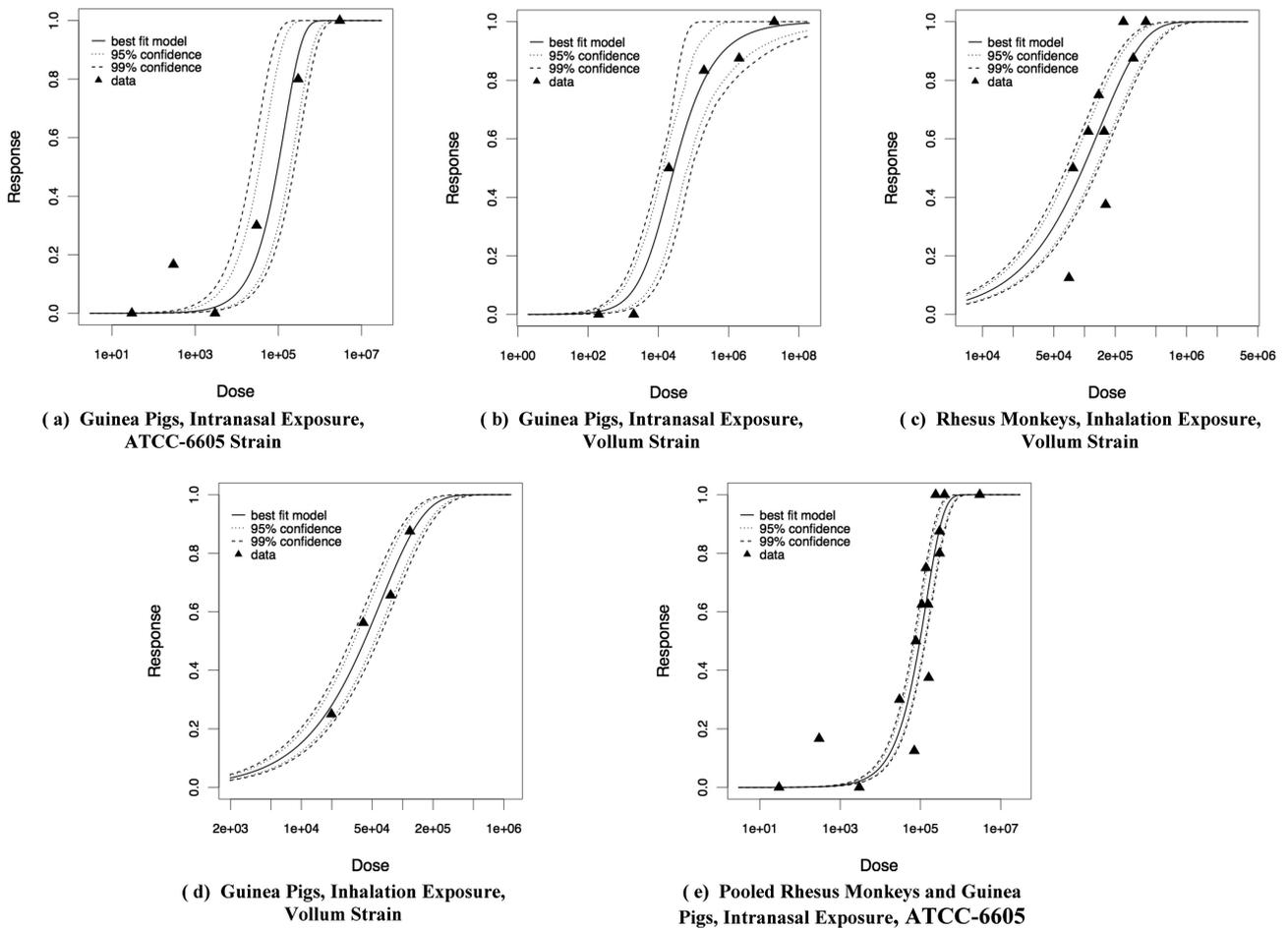


Fig. 2. Dose-response data best fits and confidence intervals.

experimental data, best fit models, and their 95% and 99% confidence intervals are presented in Fig. 2. For all data sets, the distribution of data around the best fit is described adequately by the binomial distribution (data are not overdispersed⁽³⁵⁾). This indicates that the animal models were drawn from a population with a uniform response to exposure to *B. anthracis*. By contrast, in experiments of interperitoneal exposure of mice to *B. anthracis* (mouse and *B. anthracis* strains not specified), the mouse population was determined to be comprised of two groups with differing responses.⁽³⁶⁾

Results of pooling analyses are presented in Table III. In this table, the individual data sets that were pooled are identified and the *p*-value associated with the test statistic and the conclusion is presented. The dose-response model fitting of data for guinea pig exposure to aerosols of various diameters is described below. To assess whether pooling was possible, the sum of the minimized deviances of the individual data sets was subtracted from the minimized deviance

of the best fit model for the pooled data and the value was compared with the critical chi-square distribution with degrees of freedom equal to the sum of the number of parameters used in fits of the individual data sets minus the number of parameters used to fit the pooled data set. The null hypothesis (the pooled data come from the same distribution) was rejected when the difference in optimized deviances was in excess of the critical χ^2 value. Pooling of data was possible only for:

- intranasal inhalation of ATCC and Vollum strains in guinea pigs;
- intranasal inhalation of ATCC strain in guinea pigs with inhalation exposure of Vollum strain in rhesus monkeys; and
- aerosol exposure of guinea pigs to 1 μm aerosols of the Vollum strain with aerosol exposure of guinea pigs to 3.5 μm aerosols of the Vollum strain.

Table III. Evaluation of Pooling of Data from Different Experiments

Data Set 1	Data Set 2	<i>P</i>	Conclusion
Guinea pigs, intranasal inhalation, ATCC-6605 strain	Guinea pigs, intranasal instillation, Vollum strain	0.26	Data may be pooled
Guinea pigs, intranasal inhalation, ATCC-6605 strain	Guinea pigs, aerosol exposure, 1 μm aerosol, Vollum strain	0.01	Data may not be pooled
Guinea pigs, intranasal inhalation, Vollum strain	Guinea pigs, aerosol exposure, 1 μm aerosol, Vollum strain	0.04	Data may not be pooled
Guinea pigs, intranasal inhalation, ATCC-6605 strain	Rhesus monkeys, aerosol exposure, Vollum strain	0.40	Data may be pooled
Guinea pigs, intranasal inhalation, Vollum strain	Rhesus monkeys, aerosol exposure, Vollum strain	0.0003	Data may not be pooled
Guinea pigs, aerosol exposure, 1 μm aerosol, Vollum strain	Guinea pigs, aerosol exposure, 3.5 μm aerosol, Vollum strain	0.903	Data may be pooled
Guinea pigs, aerosol exposure, 3.5 μm aerosol, Vollum strain	Guinea pigs, aerosol exposure, 4.5 μm aerosol, Vollum strain	0.024	Data may not be pooled

Inferences that may be drawn from the pooling analyses are that strain to strain variation in response associated with the ATCC-6605 and Vollum strains is relatively minor for intranasal instillation in guinea pigs and that responses to aerosols 3.5 μm in diameter and less are not dependent on aerosol diameter. No definitive conclusions can be made on interspecies extrapolation based on these results.

4.2. Variation in Response with Species and Aerosol Diameter

Numerous studies^(11,37-40) have reported that response to inhalation exposure of an aerosol of pathogenic organisms is a strong function of aerosol size. To explore the variation of mortality with aerosol size, dose-response data for guinea pigs and non-human primates exposed to homogeneous aerosol clouds with different aerosol sizes were fit with dose-response models. *B. anthracis* dose-response data for

guinea pigs exposed to aerosols of mean diameters of 1 μm, 3.5 μm, 4.5 μm, 8 μm, and 12 μm and for rhesus monkeys exposed to aerosols with mean diameter of 1 μm and 12 μm were taken from Druett *et al.*⁽¹¹⁾ In the original experimental studies, mean diameter was determined via microscopic examination of droplets sampled by direct deposition for droplets of diameter equal to or greater than 8 μm and sampled in a one-stage impactor for smaller droplet sizes. Fits to those data are summarized in Table IV. Goodness of fit was realized for all best fit models.

Variation in guinea pig and rhesus monkey LD₅₀, LD₁₀, and LD₁ with aerosol size is illustrated in Fig. 3. In Fig. 3, data for rhesus monkeys exposed to 12 μm aerosols are shifted slightly to improve visibility. For guinea pigs, there is no significant variation of LD₅₀, LD₁₀, or LD₁ with aerosol size for aerosols of 1 μm, 3.5 μm, and 4.5 μm. Pooling of data for 1 μm and 3.5 μm aerosols was possible, but 4.5 μm data could not be pooled with either of the data sets corresponding

Table IV. Best Fit Models for Data Sets with Differing Aerosol Sizes

Animal Model	Aerosol Diameter	<i>n</i> _{doses}	Best Fit Model	Parameters	Minimized Deviance	<i>P</i>
Guinea pig	1 μm	4	Exponential	<i>k</i> = 1.651 × 10 ⁻⁶	1.428	0.699
Guinea pig	3.5 μm	7	Exponential	<i>k</i> = 1.617 × 10 ⁻⁶	5.556	0.475
Guinea pig	4.5 μm	8	Beta-Poisson	α = 0.628, <i>N</i> ₅₀ = 54,710	8.645	0.195
Guinea pig	8 μm	5	Exponential	<i>k</i> = 1.460 × 10 ⁻⁶	6.726	0.151
Guinea pig	12 μm	7	Beta-Poisson	α = 0.822, <i>N</i> ₅₀ = 620,301	0.502	0.992
Rhesus monkey	1 μm	9	Exponential	<i>k</i> = 7.164 × 10 ⁻⁶	11.253	0.188
Rhesus monkey	12 μm	8	Beta-Poisson	α = 0.663, <i>N</i> ₅₀ = 1.483 × 10 ⁶	8.043	0.235

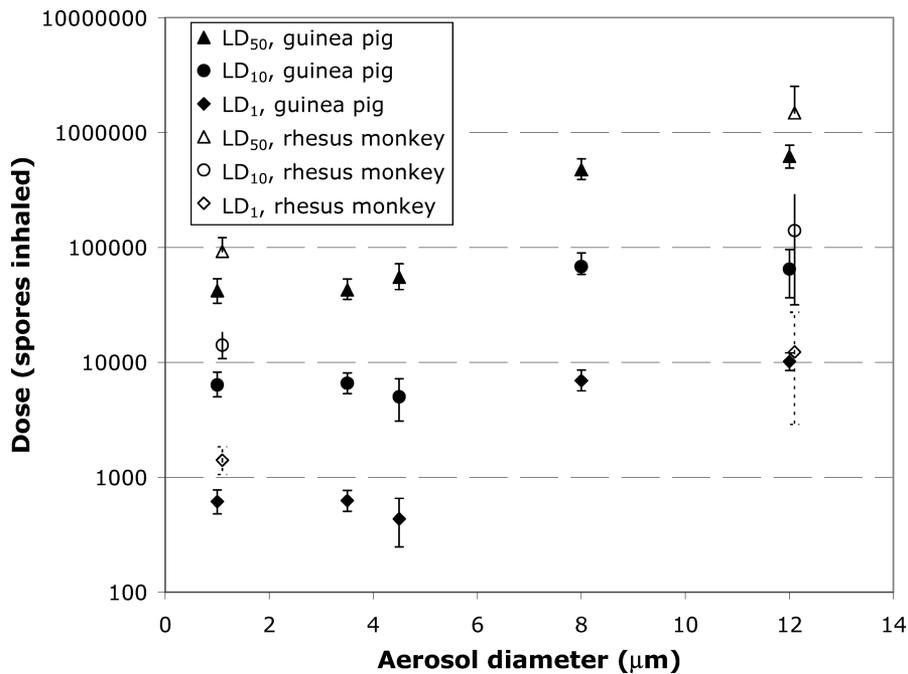


Fig. 3. Variation of *B. anthracis* lethal dose with aerosol size (95% confidence intervals indicated).

to smaller aerosol diameters. For aerosol sizes of 1 μm and 12 μm , the LD₅₀ for the rhesus monkeys increases by a factor of 16 and the LD₅₀ for the guinea pigs changes by a factor of 15. The LD₁₀ for the rhesus monkeys and guinea pigs changes by factors of 10 for both animal models for aerosol sizes of 1 μm and 12 μm .

5. SUMMARY AND DISCUSSION

Dose-response data from the open literature for inhalation exposure to *B. anthracis* were reviewed and analyzed. Relatively few complete data sets amenable to the dose-response analysis techniques used in this study were located. All dose-response data showed distinct dose-response behavior (low dispersion; apparent low host-to-host variation in susceptibility). Data were fit with exponential, beta-Poisson, and log-probit dose-response models. Three data sets were fit best with the exponential model; the other set was best fit with the beta-Poisson model.

Among data sets identified, only one (intranasal exposure of guinea pigs to the ATCC-6605 strain) could be pooled with data for rhesus monkey exposure to the Vollum strain via inhalation. The LD₅₀ for the pooled data set was 94,320 with a 95% confidence interval of (74,100, 125,060). The LD₁₀ for the pooled

data set was estimated to be 14,800 with a 95% confidence interval of (11,100, 19,600).

Dose-response data sets corresponding to different aerosol sizes were fit with dose-response models. All data for small aerosol diameters (3.5 μm and less) were best fit with the exponential dose-response model. Above 3.5 μm the exponential model fit some data sets best and the beta-Poisson model others. Aerosol diameter was found to alter the response of guinea pigs and rhesus monkeys to inhalation exposure to *B. anthracis*. For *B. anthracis*, dose-response data for aerosols of 3.5 μm and less could be pooled, indicating that the data sets corresponding to different aerosol diameters come from the same distribution. Above 5 μm , the LD₅₀ increases with increasing aerosol size. This trend of response with respect to aerosol diameter has been observed across animal models and pathogens.

In review of open literature for *B. anthracis* risk, several data gaps were identified:

- dose-response data for animal models drawn from different populations (e.g., inbred animal models and animals caught in the wild in regions where anthrax spores are believed to be present);
- dose-response data for cutaneous exposure to *B. anthracis*;

- exploration of factors altering subpopulation susceptibilities to anthrax infection and quantification of the portion of human population possessing those factors; and
- effect of the time over which a dose is administered on animal response.

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