Johns Hopkins University, Dept. of Biostatistics Working Papers

12-1-2003

Modeling the Incubation Period of Anthrax

Ron Brookmeyer
Johns Hopkins Bloomberg School of Public Health, rbrookmeyer@ucla.edu

Elizabeth Johnson
Johns Hopkins Bloomberg School of Public Health

Sarah Barry
Johns Hopkins Bloomberg School of Public Health

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Ron Brookmeyer, Elizabeth Johnson and Sarah Barry

Department of Biostatistics

Johns Hopkins Bloomberg School of Public Health

Baltimore, Maryland USA

SUMMARY

Models of the incubation period of anthrax are important to public health planners because they can be used to predict the delay before outbreaks are detected, the size of an outbreak and the duration of time that persons should remain on antibiotics to prevent disease. The difficulty is that there is little direct data about the incubation period in humans. The objective of this paper is to develop and apply models for the incubation period of anthrax. Mechanistic models that account for the biology of spore clearance and germination are developed based on a competing risks formulation. The models predict that the incubation period distribution depends critically on the rate that spores are cleared from the lung and to a lesser extent on the dose of inhaled spores. The models are used in a statistical analysis of data from an anthrax outbreak that occurred in Sverdlovsk, Russia. The analysis suggests that spores are cleared from the lung at a rate between 8% per day, and 14% per day, which is in good agreement with experimental studies of animals. The analysis suggests that at low doses, the overall median incubation period time is about 10 days, which includes a median lag of about 2 days between spore germination and onset of symptoms. Male gender and younger ages were associated with longer incubation periods as was lower dose of inhaled spores.

KEY WORDS: anthrax; competing risks; incubation period; models

Correspondence to: Ron Brookmeyer, Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe Street, Baltimore, MD 21205 USA

1. INTRODUCTION

Anthrax is of public health concern because of its potential use as a biological weapon [1]. Inhalational anthrax, which is the severest form of the disease, occurs when persons breathe anthrax spores into the lungs [2]. Considerable morbidity and mortality could result from either the accidental or intentional release of anthrax spores because spores can exist in aerosol form and be disseminated widely. Knowledge of the incubation period of inhalational anthrax is important for predicting the size of an outbreak, the delay until recognition of the outbreak, and the duration of time that antibiotics should be taken to prevent symptomatic disease [3-6]. However, there is a paucity of data about the incubation period because of the rarity of the disease. Indeed, only 18 cases of inhalational anthrax occurred in the United States in the last century. A total of 11 cases occurred in the 2001 U.S outbreak. The only other large documented outbreak in recent times occurred in Sverdlovsk, Russia in 1979 [7]. A goal of this paper is to demonstrate that mechanistic models, in combination with the available data, can help to define the entire shape of the incubation period distribution.

Anthrax is caused by the bacteria Bacillus anthracis. After spores enter the lung, they may be carried by macrophages to the mediastinal lymph nodes where they may germinate. Once the spores germinate, they multiply and produce toxins that cause symptomatic disease which if not treated rapidly is likely fatal [2, 7]. The objective of this paper is to develop statistical and mathematical models for the incubation period. A probabilistic model for the incubation period that accounts for spore dynamics is developed in section 2. The models are applied to the statistical analysis of data from a Russian anthrax outbreak in section 3. The implications of the results are discussed in section 4.

2. MODELS FOR THE INCUBATION PERIOD

2.1 Competing risks model

In this section we develop a probabilistic model for the incubation period of anthrax infection [5]. The incubation period of disease is the time from exposure to the infectious agent to the onset of disease. We define the cumulative attack probability function of disease, $F(t)$, to be the cumulative probability that a person develops disease in less than t days following exposure. However, not all persons exposed to the infectious agent will develop disease. If only a proportion p of exposed persons develops disease, then $F(t)$ is a nondecreasing function that approaches the value p as t increases. We introduce another function $F^*(t)$ which we call the incubation period distribution and is defined to be the probability that the incubation period is less than t days among the proportion p of persons who would eventually develop disease. Thus, $F(t)$ and $F^*(t)$ are related through the equation,

$$F(t) = p\, F^*(t)$$

The incubation period distribution $F^*(t)$ is the normalized version of $F(t)$, and $F^*(t)$ approaches 1 as t increases.

One of two events could happen to an anthrax spore that is inhaled into the lung. The spore could be cleared from the lung by either being expelled through the bronchus, swallowed or destroyed by macrophages. Alternatively, the spore could germinate at which point it quickly reproduces and releases toxins that cause symptomatic disease. A competing risks model can describe the dynamics of spore clearance and germination. Let the clearance rate θ represent the hazard rate or risk per unit time that a spore is cleared from the lung. Let the germination rate λ represent the hazard or risk per unit time that a spore germinates. We assume that these hazards

are constant over time, and that spore clearance and germination are independent events. Then, the probability that a spore germinates before it is cleared is

$$\int_0^\infty \lambda e^{-\lambda t} e^{-\theta t} dt = \lambda/(\lambda + \theta).$$

Consider an individual who breathes a number D spores into the lung. If we assume that each spore acts independently, and the probability $\lambda/(\lambda+\theta)$ is small, then the number of the D spores that germinate, X, can be approximated by a Poisson distribution with mean $\{D\,\lambda/(\lambda+\theta)\}$. Then, the probability that at least one spore germinates is the attack rate (AR) and is $P(X > 0) = 1 - P(X = 0)$ which from the Poisson distribution is

$$AR = 1-\exp(-D\lambda/(\lambda+\theta)). \quad (1)$$

The exponential form for the attack rate which is given by equation 1 is consistent with the empirical studies in animals [9].

The dose of spores that causes disease in P percent of the population, that is with probability $p=P/100$, is called the toxic dose TD(P) and sometimes is also called the infectious dose ID(P). We can solve for the TD(P) by setting the attack rate equal to p and solving for D. We find that

$$TD(P) = \frac{-(\lambda+\theta)\ln(1-p)}{\lambda}. \quad (2)$$

2.2 Model for the incubation period distribution

In this section we use the model developed in section 2.1 to derive expressions for $F(t)$ and $F^*(t)$ [5]. The probability that a single spore germinates within t days is

$$\int_0^t \lambda e^{-\lambda u} e^{-\theta u} du = \frac{\lambda}{\lambda+\theta}\left(1-e^{-(\lambda+\theta)t}\right).$$

Using the above expression and the Poisson distribution in a derivation analogous to that used to derived the attack rate in section 2.1, we find that the probability that at least one of the *D* spores germinates within t days is

$$F(t) = 1 - \exp\left[\frac{-D\lambda}{\lambda+\theta}\left(1-e^{-(\lambda+\theta)t}\right)\right]. \qquad (3)$$

It is seen from equation 3 that three parameters describe the cumulative attack probability function *F(t)*: the germination rate λ, the clearance rate θ, and the dose of inhaled spores. The function *F(t)* increases as either the dose increases, the germination rate increases or the clearance rate decreases. As t approaches infinity, equation 3 approaches the attack rate given by equation 1.

Animal studies can provide some information about the biological parameters θ and λ. One experimental study with rhesus macaque monkeys presented data on the declining percentages of spores that remained over time in the lung [10]. We performed a reanalysis of that data in order to obtain information about the value of the clearance rate θ. Figure 1, which is based on the rhesus macaque monkey study, shows the decreasing percentage of retained spores in the lung on a log scale as a function of time since inhalation. We fit an exponential decay model $R = \exp(-\theta t)$ where *R* is the fraction of retained spores by regressing log *R* on *t*. We did not include an intercept term because *R* must equal 1.0 when $t = 0$. We estimated that the clearance rate was $\theta = .07$ per day, that is, spores were cleared at a rate of 7% per day.

Some estimates of the germination rate λ can also be gleaned from experimental studies with animals. These studies have estimated that the dose associated with an attack rate of 50% was between 4,000 spores to over 100,000 spores [9, 11]. If we use these values in equation 1 with

the attack rate set equal to .50, $\theta = .07$ and solve for λ, we find that λ is on the order of between 5×10^{-7} and 1×10^{-5}. Thus, λ is small relative to θ, and we can make the approximation that $\lambda+\theta \approx \theta$ in equation 3.

Equation 3 is illustrated in Figure 2, which shows how the cumulative attack probability function depends on the clearance rate and dose of inhaled spores. We fixed the germination rate at $\lambda = 5 \times 10^{-6}$ and illustrated *F(t)* for various doses of spores and clearance rates. The figure illustrates that *F(t)* increases as the dose of spores increases (with the clearance rate held fixed) or as the clearance rate decreases (with the dose held fixed). The figure also illustrates that different combinations of the clearance rate and the dose will produce the same final attack rate (AR). In particular, with the approximation that $\lambda+\theta \approx \theta$, we see from equation 1 that the attack rate is determined essentially by the factor $(D\,\lambda / \theta)$.

Equation 3 becomes especially simple if we evaluate *F(t)* when the dose *D* is set equal to the *TD*(*P*). With *D=TD(P)*, equation 3 becomes

$$F(t,\, p) \,=\, 1 - \left(1-p\right)^{\left(1-\exp(-\left(\lambda+\theta\right)t)\right)} \tag{4}$$

Recalling that $\lambda+\theta \approx \theta$, we obtain from equation 4 the following remarkably simple expressions:

$$F(t,\, p) \,\approx\, 1 - \left(1-p\right)^{\left(1-\exp(-\theta t)\right)} \tag{5a}$$

$$F^{*}(t,\, p) \,\approx\, \frac{1-\left(1-p\right)^{\left(1-\exp(-\theta t)\right)}}{p} \tag{5b}$$

where we have indexed *F(t, p)* both by *t* and *p=P*/100 to emphasize that the cumulative attack probability function depends critically on the dose of inhaled spores. Although equations 5a and

5b do not explicitly involve the parameter λ, they indirectly involve λ because the dose TD(P) depends on λ.

Table 1 illustrates how the median incubation period (i.e., median of $F^*(t, p)$) depends both on the dose and the clearance rate. The median incubation period decreases if either θ or p increases. At first it may appear counterintuitive that the incubation period decreases as θ increases in light of the fact that the cumulative attack probability function decreases as θ increases. The reason that the median incubation period decreases if the clearance rate θ increases is because amongst persons who get the disease, spores will need to germinate more quickly if they are to win the race and germinate before the spores are cleared from the lung. Figure 3 is a contour plot that shows values of p and θ that yield the same median incubation period. The curves in Figure 3 are relatively flat or constant over values of TD(P) which indicates that the main determinant of the median incubation period is the clearance rate and that the dose has a considerably smaller effect on the incubation period. While dose is not a major determinant of the incubation period distribution $F^*(t, p)$, it is a major determinant of the cumulative attack probability function $F(t, p)$.

It is interesting to explore the limiting behavior of $F^*(t, p)$ at low doses. The limit of $F^*(t, p)$ as p goes to 0 from L'Hospital's rule is $\lim_{p\to 0} F^*(t, p) = 1 - e^{-\theta t}$. Thus, at very low doses, the incubation period distribution is an exponential distribution with hazard rate equal to the clearance rate. This implies that the mean incubation time can never get longer than the mean time to clearance of a single spore no matter how low the dose. This analysis suggests that in small anthrax outbreaks where the dose is relatively low, the median incubation period would be approximately $ln2/\theta$ which is 9.9 days if one uses the value θ =.07 per day estimated from the primate studies.

The derivation of equations 3 and 5 assumed that symptoms occurred immediately after germination of a single spore. While it is generally believed that the onset of symptoms occurs very rapidly following spore germination, models could be developed that account for a non-negligible period between germination and symptoms. Bacterial growth is characterized by a lag phase during which time the newly germinated cell adapts to its new environment (12). After the lag phase, an exponential phase begins when the population size of the bacteria grows exponentially and bacterial toxins are produced that cause symptoms. A simple model for the duration between germination and symptoms is an exponential distribution with hazard rate γ that we assume is independent of the germination time. Then, the incubation period distribution is obtained from the convolution of equation 5b with an exponential distribution and is

$$F^*(t,p) = p^{-1}\int_0^t \left(1-(1-p)^{\left(1-\exp(-\theta(t-s))\right)}\right)\gamma e^{-\gamma s}\,ds \qquad (6)$$

In the special case when the dose is low, the limiting distribution of equation (6) is that of the sum of two independent exponential distributions with parameters θ and γ. In this situation, the incubation period distribution $F^*(t)$ and density $f^*(t)$ are respectively [13]

$$F^*(t) = 1+\left(\frac{\theta e^{-\lambda t} - \gamma e^{-\theta t}}{\gamma-\theta}\right) \qquad (7a)$$

$$f^*(t) = \frac{\gamma\theta\left(e^{-\theta t} - e^{-\gamma t}\right)}{\gamma-\theta} \qquad (7b)$$

An important point concerning the equations 7a and 7b are that they are symmetric in θ and γ. As such the likelihood function could not provide information for distinguishing between the two parameters. However, it is believed that the time from germination to symptoms is considerably shorter than the time from exposure to anthrax spores to germination [14, 15]. In our application

of this model to the Sverdlovsk data (section 3), we estimate θ for fixed values of γ, and use a range of values for γ in a sensitivity analysis. The duration between germination and symptoms could be accounted for by models more complicated than the exponential distribution that account for both the bacterial lag and exponential phases of growth of the bacteria. However, because of the limited data available to estimate model parameters, we have chosen to model this duration by the simpler exponential model.

3. ANALYSIS OF THE SVERDLOVSK OUTBREAK

In 1979 an outbreak of inhalational anthrax occurred in the city of Sverdlovsk, Russia [7]. In this section we analyze the Sverdlovsk data using the models derived in section 2. Anthrax spores were accidentally released from an open vent of a military microbiology facility in Sverdlovsk on April 2, 1979. It is assumed that all exposures to the anthrax spores from that outbreak occurred on April 2, 1979 because anthrax spores typically do not remain suspended in air for more than a day and there is no person-to-person transmission. The data from the Sverdlovsk outbreak includes the incubation times of 70 anthrax cases [7, 16]. A public health intervention program of antibiotics and vaccine to prevent disease among exposed persons was initiated several weeks after the release of the spores. This intervention introduced a statistical complication because persons with long potential incubation periods may have received antibiotics before disease onset and thus their disease may have been prevented. As such, some persons with potentially long incubation periods may have been selectively excluded producing right truncated data. A previous statistical analysis addressed this question and found that the effects of the right truncation in the Sverdlovsk data is relatively small and accordingly in this analysis we will ignore these effects [16].

We fit the model based on equation 5b to the 70 incubation periods by maximizing the likelihood function. The log likelihood function was log L= $\sum \log f^*(t_i; p, \theta)$ where $f^*(t;p, \theta)$ is the incubation period density which from equation 5b is

$$f^*(t; p, \theta) = -\theta e^{-\theta t} p^{-1} (1-p)^{1-\exp(-\theta t)} \ln(1-p)$$

We maximized the likelihood by performing a grid search over values of θ and p. The maximum likelihood estimates were θ =.082, and p= .001. We found a 95% confidence interval for θ by inverting a likelihood ratio test and finding all values of θ that would not be rejected in a hypothesis test. The 95% confidence interval for θ was (.053, .112). We found joint 95% and 80% confidence regions for $P= p$ x 100 and θ by inverting a likelihood ratio test and it is shown in Figure 4. The figure makes clear that a wide range of *TD(P)* dose levels are consistent with the observed data. As such, although there is considerable information in this data about θ, there is relatively little statistical information about the dose level in case-only data such as the Sverdlovsk data. Indeed, the likelihood surface was relatively flat in p. However, other studies suggested the dose in Sverdlovsk was low. An epidemiological study of the Russian outbreak had suggested that less than 2% of the exposed population in Sverdlovsk were affected [7, 16].

We also fit a model that accounted for the delay between germination and symptoms. We assumed the delay followed an exponential distribution, leading to the incubation density given by equation 7b. We tried several values for γ corresponding to a median delay between germination and symptoms of 1, 2 and 3 days. We maximized the likelihood by performing a grid search over values of θ for fixed values of γ. The MLE of θ corresponding to median delay of 1, 2, and 3 days were respectively $\hat{\theta}$ = .094 (95% CI (.074 - .118)), $\hat{\theta}$ = .109 (95% CI (.085 - .138)), and $\hat{\theta}$ = .128 (95% CI (.098 - .166)). The model with median delay of 2 days appeared to give the best fit to the data based on a graphical assessment. Figure 5 displays

a histogram of the 70 incubation times from Sverdlovsk along with the estimated incubation density based on equation 7b with a median delay of 2 days between germination and symptoms.

A number of covariates were available on the Sverdlovsk cases including gender, and age (≤ 40 years versus > 40 years). In addition a surrogate measure of dose of spores was available that was based on the location that persons lived or worked relative to the location where spores were released (7). This dose variable was based on an atmospheric dispersion model that accounted for wind speed and wind directions on April 2, 1979. This model yielded contours of equal dosages of spores on a map of Sverdlovsk (7). Persons were classified into one of four dose contours (low, medium, high, and very high) based on their location at the time of the release of the spores (7).

Table 2 shows summary statistics for each of the covariates including the median incubation period. We fit a normal regression model to the log of the incubation periods of the 70 cases that included the covariates age, gender and dose contour. The predicted medians for various combinations of covariates based on the regression model are shown in Table 3. We found a significant dose effect using the dose contours derived from the atmospheric dispersion considerations in the regression analysis ($p = .01$). The predicted median incubation periods were 1.8, 1.7, and 1.4 times longer in the low, medium and high dose contours compared to the very high contour. Interestingly, a simple measure of distance from the source was not significantly associated with incubation periods. An explanation for that finding is that even if persons were close to the source, the inhaled dose could still be low if wind directions were blowing in the opposite direction. After controlling for dose, we found that males had median incubation periods roughly 1.6 times that of females (p-value =.01), and persons under the age of 40 had median incubation periods roughly 1.6 times that of older persons (p-value =.02). One

interpretation of these results is that the clearance rate parameter θ depends on both gender and age. However, an alternative explanation is that the dose contour variable was not a satisfactory surrogate for dose of inhaled spores, and accordingly dose was not adequately controlled for in the analysis. The dose contour variable was based only on geographic and meteorological considerations but other factors affect the inhaled dose including a person's respiratory rate and capacity as well as whether they were indoors or outdoors at the time of the release of spores. Accordingly, the interpretation of how covariates affect the dynamics of spore clearance and germination should be interpreted cautiously from this analysis.

4. DISCUSSION

The objective of this paper was to develop statistical and mathematical models for the incubation period of anthrax that address the biology of the infection. There is relatively little empirical data in humans about the incubation period of inhalational anthrax because the disease is rare. Accordingly, we must rely on mechanistic models together with whatever empirical data is available. The models developed in section 2 account for the dynamics of spore clearance and spore germination. Using the estimate of the clearance rate derived from studies in animals, we were able to predict the incubation period of disease observed in humans in a low dose outbreak such as in Sverdlovsk. The median incubation period based on the competing risks probabilistic model with θ=.07 per day was 9.9 days which was in excellent agreement with the actual observation of 10.0 days in the Sverdlovsk outbreak (Table 2). The concordance between the predictions based on the probabilistic model and the human outbreak in Sverdlovsk corroborates both the model and the value of θ.

When we fit the models directly to the Sverdlovsk outbreak we estimated the clearance rate of .084 per day which again was in excellent concordance with the independent estimate obtained from the primate studies. When we fit the model that included a delay between germination and symptoms, we found that a median delay between germination and symptoms of 2 days yielded a good fit to the Sverdlovsk data

The entire shape of the incubation distribution is of interest, not just the median or mean. The left tail of the curve is critical for predicting how long it would be before symptomatic cases surface which is important for determining the delay before an outbreak is recognized. The right tail of the incubation period distribution is important for predicting the ultimate size of an outbreak and how long persons should remain on antibiotics. Because of the rarity of anthrax, currently available empirical data alone without mechanistic models cannot accurately resolve the shape of the incubation distribution. The mechanistic models developed in this paper are useful for piecing together the limited data from animals and humans to develop a coherent model for the incubation period of anthrax.

Figures

Figure 1: Fraction of spores remaining in lungs of Rhesus Macaque monkeys by time since inhalation based on data in Henderson (10).

Figure 2: Cumulative attack probability function $F(t, p)$ by time since exposure assuming $\lambda = 5 \times 10^{-6}$ for different values of the clearance rate θ and dose of spores D

Figure 3: Contours of the clearance rate θ and $TD(P)$ that give constant values of the median incubation period.

Figure 4: Joint 95% and 80% confidence regions for P and θ based on analysis of the Sverdlovsk data using model given by equation 5.

Figure 5: Histogram of incubation periods from Sverdlovsk outbreak and estimated incubation period density based on equation (7b) with a median delay between germination and symptoms of 2 days ($\gamma = .346$/day), and estimated clearance rate $\hat{\theta} = .109$/day.

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Table I. Median incubation periods (in days) by clearance rate θ and dose of inhaled spores. The *TD(P)* is the dose of spores to ultimately cause disease in P% of the population.

	θ			
	.05	.07	.11	.15
TD(1)	13.8	9.9	6.3	4.6
TD(10)	13.3	9.5	6.1	4.5
TD(25)	12.5	8.9	5.7	4.1
TD(50)	10.7	7.7	4.9	3.6

Table II. Summary statistics of incubation periods (in days) among cases in the Sverdlovsk outbreak.

		N[1]	Mean	(SD)	Median	Min	Max
All subjects		70	12.19	(8.67)	10.0	2	40
Gender	Male	51	13.71	(9.42)	10.0	4	40
	Female	19	8.11	(4.20)	7.0	2	15
Dose contour	Low	8	14.75	(9.79)	12.0	6	35
	Medium	18	12.72	(8.80)	10.0	2	37
	High	15	11.73	(8.20)	10.0	2	27
	Very high	10	9.50	(6.69)	7.5	3	23
Distance	> 2km	27	13.22	(9.74)	10.0	2	37
	1-2km	7	11.43	(4.47)	12.0	7	20
	< 1km	15	10.27	(7.20)	8.0	3	26
Age	≤ 40	18	16.89	(11.85)	11.5	4	40
	> 40	52	10.56	(6.67)	9.5	2	32

[1]Sample size

Table III. Predicted median incubation periods based on normal regression model of the log incubation periods of Sverdlovsk outbreak

	Median incubation period in days (SE)											
	≤ 40 years						> 40 years					
	Male			Female			Male			Female		
Dose contour	N	Median	(SE)	N	Median	(SE)	N	Median	(SE)	N	Median	(SE)
Low	2	20.03	(5.03)	0	11.91	(3.73)	4	12.53	(2.78)	2	7.45	(1.88)
Medium	4	18.09	(3.57)	1	10.76	(2.69)	7	11.31	(1.97)	6	6.73	(1.23)
High	3	15.31	(3.27)	0	9.10	(2.58)	8	9.57	(1.62)	4	5.69	(1.17)
Very high	4	10.93	(2.38)	0	6.50	(1.96)	5	6.84	(1.40)	1	4.06	(1.03)

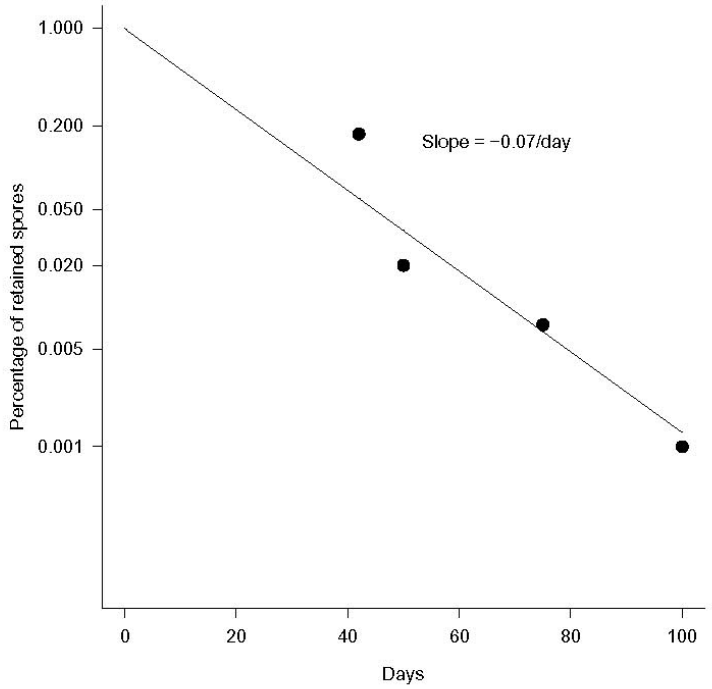

Figure 1: Fraction of spores remaining in lungs of Rhesus Macaque monkeys by time since inhalation based on data in Henderson (10)

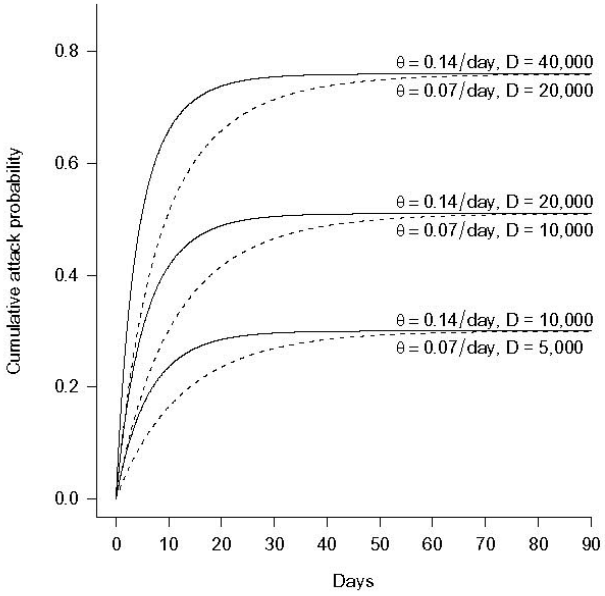

Figure 2: Cumulative attack probability function $F(t, p)$ by time since exposure assuming $\lambda = 5 \times 10^{-6}$ for different values of the clearance rate θ and dose of spores D

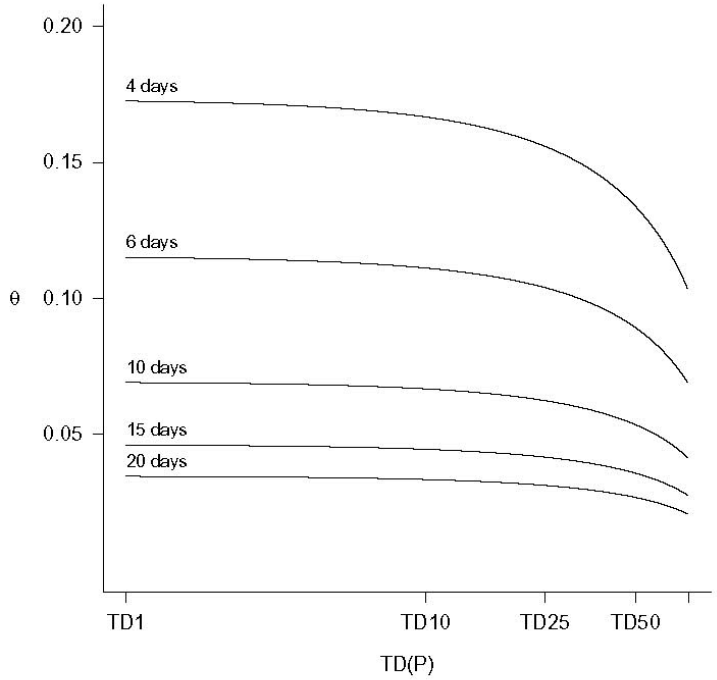

Figure 3: Contours of the clearance rate θ and $TD(P)$ that give constant values of the median incubation period

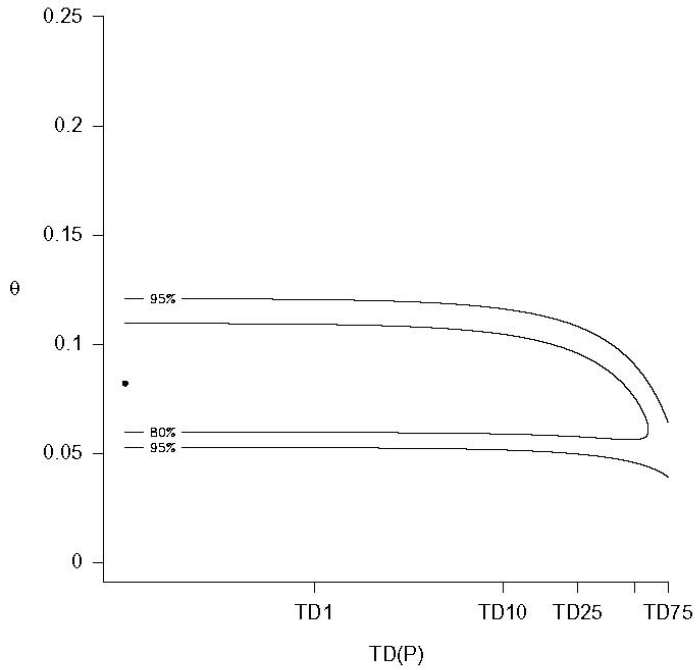

Figure 4: Joint 95% confidence region for P and θ based on analysis of the Sverdlovsk data using model given by equation 5

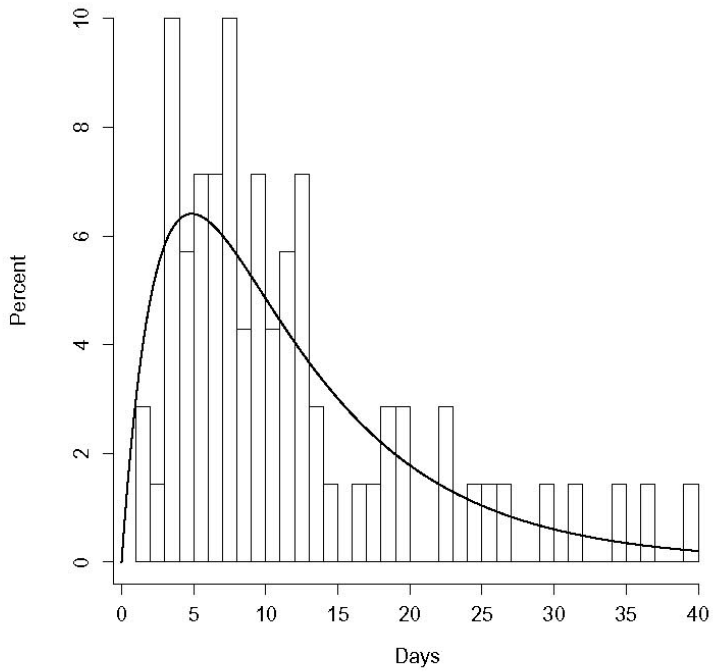

Figure 5: Histogram of incubation periods from Sverdlovsk outbreak and estimated incubation period density based on equation (7b), with a median delay between germination and symptoms of 2 days ($\gamma = 0.346$/day), and estimated clearance rate of $\hat{\theta} = .109//\text{day}$