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ORIGINAL ARTICLE

Incorporating time postinoculation into a dose–response model of *Yersinia pestis* in mice

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Keywords
chi-squared, dose–response model, maximum likelihood estimation, mice, plague, survival, time postinoculation, *Yersinia pestis*.

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Abstract

Aims: To develop a time-dependent dose–response model for describing the survival of animals exposed to *Yersinia pestis*.
Methods and Results: Candidate time-dependent dose–response models were fitted to a survival data set for mice intraperitoneally exposed to graded doses of *Y. pestis* using the maximum likelihood estimation method. An exponential dose–response model with the model parameter modified by an inverse-power dependency of time postinoculation provided a statistically adequate fit to the experimental survival data. This modified model was verified by comparison with prior studies.
Conclusions: The incorporated time dependency quantifies the expected temporal effect of *in vivo* bacteria growth in the dose–response relationship. The modified model describes the development of animal infectious response over time and represents observed responses accurately.
Significance and Impact of the Study: This is the first study to incorporate time in a dose–response model for *Y. pestis* infection. The outcome may be used for the improved understanding of *in vivo* bacterial dynamics, improved postexposure decision making or as a component to better assist epidemiological investigations.

Introduction

Plague is caused by *Yersinia pestis*, a Gram-negative, facultative anaerobic, bipolar-staining, rod-shaped bacterium belonging to the family Enterobacteriaceae (Perry and Fetherston 1997). Plague may be manifested in three forms: bubonic, pneumonic and septicaemic (Lathem *et al.* 2005). An outbreak of plague in western China from September to October 2004 (Danzhou and Ding 2006) was the latest demonstration that plague continues to be a disease of concern.

While over 200 mammalian species have been reported to be naturally infected with *Y. pestis*, rodents are the most important hosts for plague (Perry and Fetherston 1997). Currently, most human plague cases in the world are classified as sylvatic plague, namely infection from rural wild animals such as mice, chipmunks, squirrels, gerbils, marmots, voles and rabbits (Christie 1982; Gage *et al.* 1992; Craven *et al.* 1993).

Transmission between rodents is achieved by their associated fleas from the infected blood of the host. The organism is not transovarially transmitted from flea-to-flea, and artificially infected larvae clear the organism within 24 h. Therefore, maintenance of plague in environment is dependent upon cyclic transmission between fleas and mammals (Bibikova 1977; Perry and Fetherston 1997).

Substantial early studies (Goldberg *et al.* 1954; Ehrenkranz and Meyer 1955; Holdenried and Quan 1956; Speck and Wolochow 1957; Lathem *et al.* 2005) have been conducted on the animal dose response for *Y. pestis* and the low LD_{50} reported indicates that *Y. pestis* is highly infectious. However, none of the prior studies reported the incubation time distributions for plague. Therefore it is desirable to use animal survival data from plague experiments for developing an understanding of the dose–response characteristic to *Y. pestis* infection over time. The results of this study are aimed at developing a

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time-dependent dose–response model for describing and predicting *Y. pestis* infection.

Method

Overview

This study consisted of the following steps:
1. selection and characterization of survival data sets for analysis
2. analysis of survival data via 'classical' dose–response modelling techniques
3. development of empirical time-dependent dose–response models via analysis of survival data
4. fitting of survival data with empirical dose–response models and selection of the best model
5. verification of the best fit model via fitting of two additional dose–response data sets.

Source of data

The data used for development of a time-dependent dose–response model in the current study were drawn from a prior study (Rogers *et al.* 2007). The survival data presented in Table 1 were obtained from personal communication with J.V. Rogers (personal communication, Battelle Memorial Institute).

In the study by Rogers *et al.* (2007), *Y. pestis* CO92 was cultured in 250 ml of heart infusion broth at 26°C on a rotary shaker at 250 rev min^{-1} prior to use. After 24 h of incubation, culture aliquots were diluted with sterile Dulbecco's phosphate-buffered saline containing 0·01% gelatine to achieve target concentrations. Balb/c mice were injected intraperitoneally with the above sterile diluent (N = 10) and all target doses of *Y. pestis* (N = 10–20 per dose) and monitored for a period of 14 days to assess mortality.

Determination of the best model among classical dose–response models

Exponential, beta-Poisson and log-probit models have been used widely for risk assessment (Haas *et al.* 1999), and in this analysis they were evaluated for fit. The equations of exponential, beta-Poisson and log-probit models are shown as eqns (1)–(3) respectively.

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

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

$$P(d) = \varphi\left(\frac{1}{q_1} \times \log\left(\frac{d}{q_2}\right)\right) \tag{3}$$

In eqns (1)–(3), d is dose, k, N_{50}, α, q_1 and q_2 are parameters of the distributions, and φ denotes the cumulative normal distribution.

Maximum likelihood estimation (MLE) as described by Haas *et al.* (1999) was implemented into the R programming language (http://www.r-project.org, accessed on 8 August 2007) to fit these models to dose–response data. In the case of the exponential and beta-Poisson models the Broyden–Fletcher–Goldfarb–Shanno algorithm was used for optimization, and the Nelder–Mead algorithm was used for the log-probit model (Haas and Jacangelo 1993; Haas *et al.* 1999). By fitting the above-mentioned models with data in Table 1, their parameters and deviances were determined. To determine the goodness of the fits, the deviances were compared to the critical values of the chi-squared distribution at a 95% confidence level $\chi^2_{0\cdot95,df}(\mathrm{df} = m - n)$,where df is the degree of freedom, m is the number of doses, n is the number of parameters. To determine the best model, the differences of deviances of candidate models were compared to the critical values of chi-squared distribution at a 95% confidence level$\chi^2_{0\cdot95,\Delta\mathrm{df}}$, where Δdf is the difference of their degrees of freedom.

Identification of the time dependency of parameters

Since responses of mice to *Y. pestis* vary not only with the dose, but also with the time postinoculation (TPI), it is desirable to incorporate the factor of time into the classical model to describe the response pattern comprehensively.

Table 1 Response of Balb/c mice after interperitoneal exposure to graded doses of *Yersinia pestis* CO92 strain (Rogers *et al.* 2007)

		Number of deaths at indicated time post inoculation (days)														
Number of mice	Dose (CFU)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
20	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	8	0	0	0	1	2	1	0	0	0	1	0	0	0	0	0
20	26	0	0	0	1	6	3	2	0	1	0	0	0	0	0	0
10	74	0	0	0	4	5	0	0	0	0	0	0	0	0	0	0
10	257	0	0	0	2	8	*									

*All animals in dose group dead.

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For this reason, the parameter estimates in the best model were plotted against TPI via regression function implemented in the R programme to verify whether there is a discernable trend.

Fitting time-dependent dose–response data using MLE

Denoting the cumulative probability distribution for number of deaths occurring at dose d_i and at time t_j after inoculation as $F(d_i, t_j)$, the predicted mortality of animals among surviving animals demonstrating a response during the time period t_{j-1} to t_j is

$$\hat{\pi}_{i,j} = \frac{F(d_i, t_j) - F(d_i, t_{j-1})}{1 - F(d_i, t_{j-1})} \tag{4}$$

The probability of observing p positive responses among surviving animals during the time period j to j+1 is

$$P_{i,j}(p_{i,j}|\hat{\pi}_{i,j}) = \binom{n_{i,j}}{p_{i,j}} \hat{\pi}_{i,j}^{p_i} (1 - \hat{\pi}_{i,j})^{n_{i,j} - p_{i,j}} \tag{5}$$

where $p_{i,j}$ is the number of positive responses observed during time period j to j+1, $n_{i,j}$ is number of surviving animals at the beginning of time period.

The constants in $F(d_i, t_j)$, were determined using MLE. The likelihood function for the set of observations made over j days for i dose groups is

$$\begin{aligned} L &= \prod_{j=1}^{m_{\text{times}}} \prod_{i=1}^{m_{\text{doses}}} P_{i,j}(p_{i,j}|\hat{\pi}_{i,j}) \\ &= \prod_{j=1}^{m_{\text{times}}} \prod_{i=1}^{m_{\text{doses}}} \binom{n_{i,j}}{p_{i,j}} \hat{\pi}_{i,j}^{p_{i,j}} (1 - \hat{\pi}_{i,j})^{n_{i,j} - p_{i,j}} \end{aligned} \tag{6}$$

where m_{doses} is the total number of dose groups and m_{times} is the number of time periods during which observations were made.

The corresponding deviance is

$$\begin{aligned} Y &= -2 \ln LR \\ &= -2 \sum_{j=1}^{m_{\text{times}}} \sum_{i=1}^{m_{\text{doses}}} \left[p_{i,j} \ln\left(\frac{\hat{\pi}_{i,j}}{\pi_{i,j}^{\circ}}\right) + (n_{i,j} - p_{i,j}) \ln\left(\frac{1 - \hat{\pi}_{i,j}}{1 - \pi_{i,j}^{\circ}}\right) \right] \end{aligned} \tag{7}$$

This expression is nearly the same as that used in fitting classical dose–response data in which only the long-term endpoint is known (Haas *et al.* 1999). The differences between this expression and the expression normally used in dose–response modelling are that there is a double sum in the deviance expression, and that p is the number of positive responses in a time period rather than at the end of observations.

The minimized deviances of the modified and original models were determined using MLE. To test if a more complex model with more parameters provides a statistically better fit compared with the original one, the improvement to the deviance provided by the additional parameters was compared with the chi-squared distribution at 95% confidence level $\chi^2_{0\cdot95,\Delta\text{df}}$ (Haas *et al.* 1999).

Verification of the modified dose–response model

The modified model with time dependency was verified by comparison with observed dose–response data from two previous studies.

A response data set of Albino mice to the doses of *Y. pestis* was drawn from the early work of Holdenried and Quan (1956) and is shown in Table 2. In this work, the Alexander strain of *Y. pestis* was grown for 24 h at 28°C in brain–heart infusion broth and dilutions of the broth from 10^{-1} to 10^{-8} in 1% peptone water prepared. Each rodent was inoculated intracutaneously into a shaven area over the right thigh. A predicted response by the modified model was compared with the observed response.

A survival response data set of guinea pigs exposed to the *Y. pestis* L.37 strain was drawn from the work of Druett *et al.* (1956) and is presented in Table 3. In this work, bacterial suspensions were prepared from growth on de-ionized casein partial hydrolysate medium incubated at 28°C. The suspension was then diluted in phosphate buffer (pH 7·6) to give the required number of organisms per millilitre. Guinea pigs weighing 350–450 g were exposed to 12 μm diameter particles carrying various amounts of *Y. pestis* organisms via the inhalation route. The percentages of deaths in Table 3 were converted into cumulative fraction mortality, with which the modified

Table 2 Response of Albino mice exposed to the dose of *Yersinia pestis* Alexander strain via intracutaneous route (Holdenfried and Quan 1956)

TPI (days)	Dose (CFU)	Number of deaths	Number of survivors
17	1	0	11
17	5	4	12
17	10	14	2
17	100	15	1
17	1000	17	0
17	10 000	16	1
17	100 000	6	0
17	1 000 000	6	0

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Table 3 Response of guinea pigs after exposure to aerosols of 12 μm particles containing organisms of *Yersinia pestis* L.37 strain (Druett *et al.* 1956)

Range of doses* (10^3 organisms)	Percentage of deaths at indicated TPI (days)																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
0–20	0	0	11	41	26	11	6	6	0	0	0	0	0	0	0	0	0
20–50	0	0	13	54	13	10	4	8	0	0	0	0	0	0	0	0	0
50–100	0	0	9	58	18	7	4	2	2	0	0	0	0	0	0	0	0
100–200	0	0	28	43	7	8	1·4	4	3	4	0	0	1·4	0	0	0	0
200–500	0	0	27	57	6	6	0	0	0	1·5	0	0	0	0	0	0	3
500–1000	0	0	38	40	10	4	4	4	0	0	0	0	0	0	0	0	0

*Doses are based on data from the original work assuming a respiratory minute volume of 0·1 l min^{-1} (Kleinman and Radford 1964).

dose–response model was compared using the nonlinear least-squares (NLS) method.

Results

Fits of the classical models to survival data

By applying the MLE method implemented in the R language, exponential, beta-Poisson and log-probit models were fitted to the data in Table 1, and the results are shown in Table 4. $\chi^2_{0\cdot95,\mathrm{df}}$ was obtained for each TPI from the chi-squared table under the corresponding degree of freedom and confidence level. The deviances and differences of deviances were compared to the values of the chi-squared distribution at a 95% confidence level. Since minimized deviances are all less than $\chi^2_{0\cdot95,\mathrm{df}}$, the models provided an acceptable degree of fit. Since the differences of deviances between the one-parameter exponential model and the two-parameter beta-Poisson and log-probit models were less than $\chi^2_{0\cdot95,1}(\Delta df = 1)$, the simpler exponential model is preferred.

Incorporating time dependency into dose–response models

In the exponential model, the k parameter is the probability that a single micro-organism can survive, replicate and initiate infection. A single micro-organism, having survived, can replicate and colonize, and this process will increase the probability that a response (morbidity or mortality) occurs over time. Hence it is reasonable to expect that as TPI increases, the risk will increase. To quantify this in the exponential model, it is plausible to regard k as a time-dependent parameter.

Estimates for the exponential parameter k for individual days were plotted as a function of TPI and trends were assessed via nonlinear regression using R. Certain relationships between the parameter and inverse TPI (1/TPI) appeared to provide acceptable fits to the trend. Fits for two inverse models were plotted along with parameter estimates in Fig. 1. The two inverse models (solid line and dashed line) provided reasonable fits to the data for TPI >3 days but provided negative values of the parameter as TPI goes to zero. Noting that no deaths were observed in the experimental data before day 3, the following time-dependent dose–response models were proposed for evaluation:

$$P(d) = 1 - \mathrm{e}^{-k(\mathrm{TPI})d} \quad (0 \le P(d) \le 1, k \ge 0) \tag{8}$$

where, for the inverse model the expression for k is

$$k(\mathrm{TPI}) = \max\left(\frac{k_0}{\mathrm{TPI}} + k_1, 0\right) \quad (\mathrm{TPI} \neq 0) \tag{9}$$

where k_0 and k_1 are parameters to be determined using MLE; for the inverse-power model, the expression for k is

$$k(\mathrm{TPI}) = \max\left(\frac{k_0}{\mathrm{TPI}^{k_2}} + k_1, 0\right) \quad (\mathrm{TPI} \neq 0) \tag{10}$$

where k_0, k_1 and k_2 are parameters to be determined using MLE. Noting that as TPI becomes very large, the modified model becomes the exponential model.

One additional time dependency of k was evaluated in this study. According to the gamma-distributed incubation time proposed by Teunis *et al.* (1999), an alternative expression for k is given by

$$k(\mathrm{TPI}) = k_0 F(\mathrm{TPI}; r, \beta) \tag{11}$$

where k_0 is a constant; $F(\mathrm{TPI}; r, \beta)$is the cumulative distribution function of gamma distribution; r is the shape parameter and β is the scale parameter.

The variation with time in the parameter k, which is equal to the probability that an ingested organism produces an infectious focus (response), does not imply that

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Table 4 Dose–response model parameter estimates for response noted at the end of each day from the experiment of interperitoneal exposure of mice to *Yersinia pestis* (Rogers *et al.* 2007)

TPI (days)	Number of doses	Model	Parameters	Minimized deviance	$\chi^2_{0\cdot95,df}$	Acceptable fit?	ΔY	$\chi^2_{0\cdot95,1}$
3	5	Exp	k = 0·00224	7·066	9·488	Yes		
		B-P	α = 0·153, N_{50} = 2395·247	4·213	7·815		2·853	3·843
		L-P	q_1 = 2·600, q_2 = 799·672	4·519	7·815		2·547	3·843
4	5	Exp	k = 0·0206	3·068	9·488	Yes		
		B-P	α = 418435·9, N_{50} = 33·603	3·068	7·815		0	3·843
		L-P	q_1 = 0·979, q_2 = 28·762	2·322	7·815		0·746	3·843
5	5	Exp	k = 0·0268	2·301	9·488	Yes		
		B-P	α = 78867·4, N_{50} = 25·902	2·301	7·815		0	3·843
		L-P	q_1 = 1·008, q_2 = 22·475	1·107	7·815		1·194	3·843
6	5	Exp	k = 0·0306	2·707	9·488	Yes		
		B-P	α = 61225·3, N_{50} = 22·645	2·707	7·815		0	3·843
		L-P	q_1 = 0·962 q_2 = 19·841	0·557	7·815		2·15	3·843
7	5	Exp	k = 0·0306	2·707	9·488	Yes		
		B-P	α = 61225·3, N_{50} = 22·645	2·707	7·815		0	3·843
		L-P	q_1 = 0·962 q_2 = 19·841	0·557	7·815		2·15	3·843
8	5	Exp	k = 0·0327	3·240	9·488	Yes		
		B-P	α = 51016·4, N_{50} = 21·171	3·240	7·815		0	3·843
		L-P	q_1 = 0·935, q_2 = 18·666	0·562	7·815		2·678	3·843
9	5	Exp	k = 0·0345	3·116	9·488	Yes		
		B-P	α = 78129·75, N_{50} = 20·116	3·116	7·815		0	3·843
		L-P	q_1 = 0·989, q_2 = 17·590	0·885	7·815		2·231	3·843
10	5	Exp	k = 0·0345	3·116	9·488	Yes		
		B-P	α = 78129·75, N_{50} = 20·116	3·116	7·815		0	3·843
		L-P	q_1 = 0·989, q_2 = 17·590	0·885	7·815		2·231	3·843
11	5	Exp	k = 0·0345	3·116	9·488	Yes		
		B-P	α = 78129·75, N_{50} = 20·116	3·116	7·815		0	3·843
		L-P	q_1 = 0·989, q_2 = 17·590	0·885	7·815		2·231	3·843
12	5	Exp	k = 0·0345	3·116	9·488	Yes		
		B-P	α = 78129·75, N_{50} = 20·116	3·116	7·815		0	3·843
		L-P	q_1 = 0·989, q_2 = 17·590	0·885	7·815		2·231	3·843
13	5	Exp	k = 0·0345	3·116	9·488	Yes		
		B-P	α = 78129·75, N_{50} = 20·116	3·116	7·815		0	3·843
		L-P	q_1 = 0·989, q_2 = 17·590	0·885	7·815		2·231	3·843
14	5	Exp	k = 0·0345	3·116	9·488	Yes		
		B-P	α = 78129·75, N_{50} = 20·116	3·116	7·815		0	3·843
		L-P	q_1 = 0·989, q_2 = 17·590	0·885	7·815		2·231	3·843

Exp denotes exponential, B-P denotes beta-Poisson, L-P denotes log-probit, and ΔY is the difference of deviances between one- and two-parameter models.

the ultimate ability of an individual organism to cause infection is changed. Rather, $k(T)$ is the probability that an organism survives, multiplies and causes a response at or before time T.

Comparison of time-dependent dose–response models

The modified exponential models (eqns 8–11) were fitted to the survival data presented in Table 1 using MLE. The parameter estimates and the minimized deviances are listed in Table 5. The three-parameter exponential model with inverse-power TPI dependency proved to be the best model among the candidate models. It provided the lowest deviance, and gave a statistically significant improvement in fit over the two-parameter inverse model by reducing the deviance by more than $\chi^2_{0\cdot95,1}$. It also provided a lower deviance compared with the sum of deviances of original exponential model on individual days. The exponential model with gamma-distributed TPI dependency showed a statistically acceptable fit but did not provide the lowest deviance. Bootstrap analysis (Haas *et al.* 1999) using R with 1000 iterations, as shown in Fig. 2, shows that the parameters of the exponential inverse-power model and their ratio were bounded tightly by the confidence intervals.

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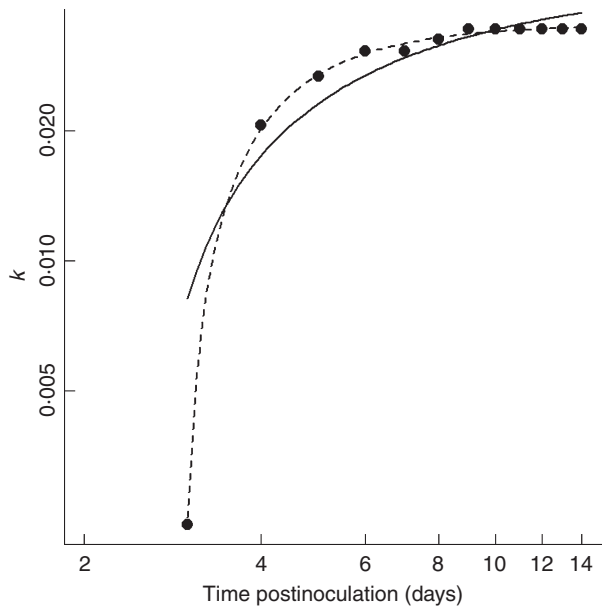

Figure 1 Trend line for exponential model parameter estimates as a function of TPI. ● denotes parameter k; (——) represents the model $k = ((k_0/\mathrm{TPI}) + k_1)$; (- - - -) represents the model $k = ((k_0/\mathrm{TPI}^{k_2}) + k_1)$.

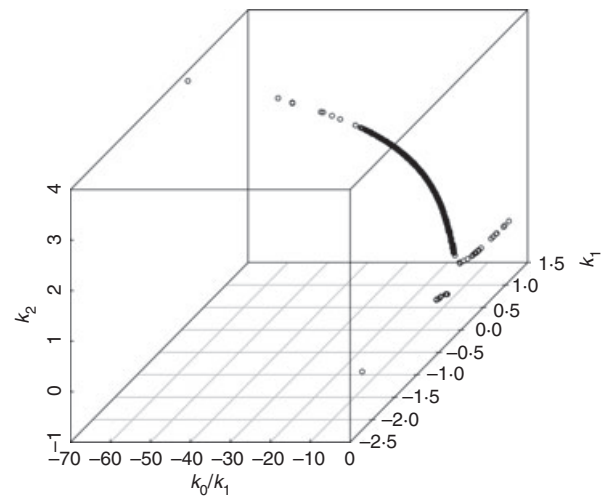

Figure 2 Bootstrap of the optimal parameters of exponential dose–response model with inverse-power TPI dependency fitted to survival data (Rogers *et al.* 2007).

The exponential inverse-power model was plotted to compare with the observed mortalities. The clear effects of the modification of the model on the different TPI groups and dose groups are shown in Figs 3 and 4 respectively. It can be seen that the modified exponential model is closely aligned with the data.

Comparison and verification between the modified exponential model and early studies

Figure 5 shows the comparison between the *Y. pestis* dose–response data drawn from the Holdenfreid and Quan (1956) study (Table 2) and the predicted curve. The curve was plotted according to the exponential inverse-power model with TPI equal to 17 and with the optimal parameter estimates in Table 5. As shown in Fig. 5, although with different strains of *Y. pestis* and mice, the modified model well predicted the observed experimental response.

As shown in Fig. 6, the exponential inverse-power model was fitted to the survival data of guinea pigs (Druett *et al.* 1956) using NLS. It can be seen that the modified model closely represents the survival reported in the independent study, showing adequate model flexibility.

Discussion

It is well known that infection is a time-dependent process (Williams 1965), and phenomenological responses of animals to bacteria vary not only with the initial dose of micro-organisms, but also with the TPI, i.e. incubation time, with infectious agents. TPI is therefore one of the most important factors required for better describing or predicting the long-term effects of infectious diseases. For

Table 5 Optimal parameters and minimized deviances for time-dependent dose-response models fitted to survival data of mice exposed to *Yersinia pestis* (Rogers *et al.* 2007)

Model	Data group	Number. of parameters	Minimized deviance	$\chi^2_{0\cdot95,\mathrm{df}}$	Acceptable fit?
Exponential with inverse-power dependency	Data pooled for 14 days (day 1 to day 14)	3 (k_0 = −0·500, k_1 = 0·0355, k_2 = 2·467)	22·515	75·624	Yes
Exponential with inverse dependency	Data pooled for 14 days (day 1 to day 14)	2 (k_0 = −0·145, k_1 = 0·0506)	27·471	76·778	Yes
Exponential with gamma-distributed dependency	Data pooled for 14 days (day 1 to day 14)	3 (k_0 = 0·0368, r = 15·411, β = 3·408)	31·929	75·624	Yes
Exponential without dependency	Data for individual days (day 3 to day 14)	12	39·785	65·171	Yes

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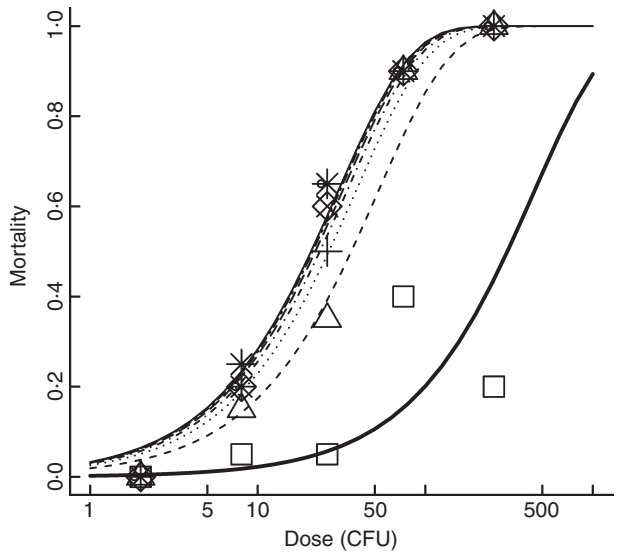

Figure 3 Exponential dose–response model with inverse-power TPI dependency (curves) compared to observed mortalities against doses (points) from the study of Rogers *et al.* (2007). (□, ——) day 3, (△, - - -) day 4, (+, ·····) day 5, (×, -·-·) day 6, (◇, – – ·) day 7, (○, –·–) day 8, (*, ——) day 9.

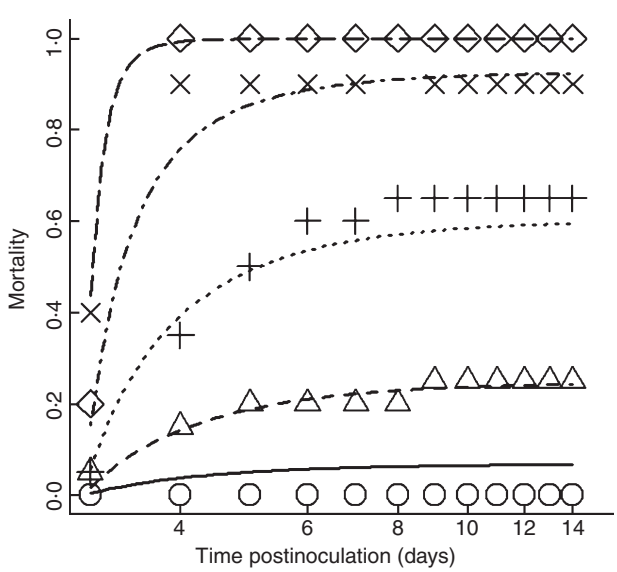

Figure 4 Exponential dose–response model with inverse-power TPI dependency (curves) compared to observed mortalities against TPI (points) from the study of Rogers *et al.* (2007). (○, ——) 2 CFU, (△, ----) 8 CFU, (+, ·····) 26 CFU, (×, -·-·) 74 CFU, (◇, – – ·) 257 CFU.

chemicals, the received dose is the total amount available to cause a response. However, micro-organisms have the ability to replicate, thus a different aspect needs to be considered in microbial risk assessment, not only the ability of the initial dose to survive but the extent to which it multiplies in the host species. This resulting amplified

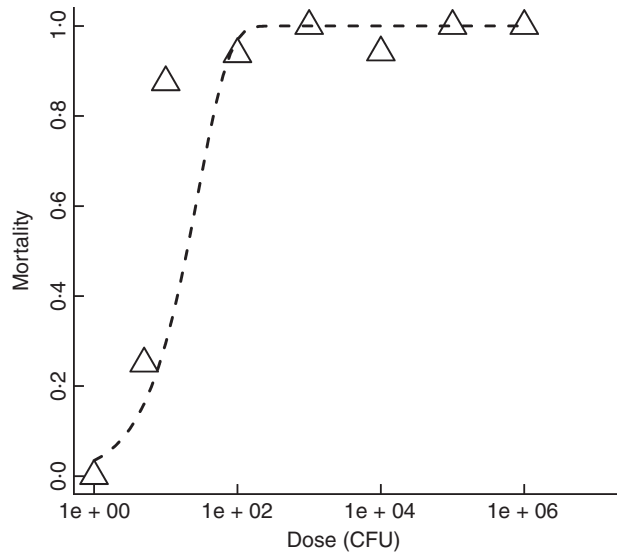

Figure 5 Predicted dose–response using the modified exponential model compared to the observed response from the study of Holdenfried and Quan (1956). (Δ), observed response; (- - - -) predicted response.

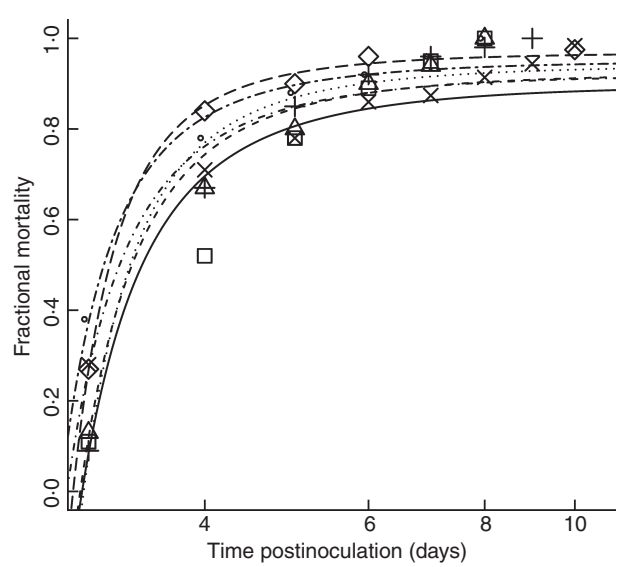

Figure 6 Comparison between exponential dose–response model with inverse-power TPI dependency (curves) and the survival response data (points) from the study of Druett *et al.* (1956). (□, ——) 0–20 (10^3 organisms), (△, - - - -) 20–50 (10^3 organisms), (+, ...) 50–100 (10^3 organisms), (×, -·-·) 100–200 (10^3 organisms), (◇, – – ·) 200–500 (10^3 organisms), (○, –·–) 500–1000 (10^3 organisms).

body burden may be the ultimate cause of the biological effect. To describe this issue comprehensively, it is desirable to incorporate the factor of time into the classical dose–response models. In this study, the incorporation of TPI yielded a modified exponential model capable of

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describing pooled time-dependent survival data and provided significant improvements to the fits.

An advantage of modifying the exponential model to include TPI effects is that the new model retains positive features of the exponential model including a mechanistic basis and linearity at low dose, which is similar to the beta-Poisson model. The log-probit model does not have a strong biological basis and does not exhibit linearity at low dose. Although the gamma-distributed dependency did not provide the best fit in this study, it still allows the possibility for future study that parameters of dose–response models could be modified by certain probability distribution functions of TPI with stronger theoretical basis over pure empirical models.

As mentioned previously, dose–response data taken from studies with different species of rodents (mice and guinea pigs) and inoculation routes (intraperitoneal, intracutaneous and inhalational) were used in this study in order to evaluate the robustness of the proposed time-dependent dose–response model. Despite their differences, the data sets were well described by the modified model with the same functional form. This result indicates that the model is of an appropriate form and flexibility to describe *Y. pestis* infection occurring in rodent species for which the survival dose–response data are currently available. In other words, host and inoculation route differences are manifest only on the parameter values and not on the functional form. Further study on the impact of hosts from different orders of mammals (e.g. nonhuman primates) and inoculation routes on the model robustness is currently in progress.

The doses shown in Table 3 were estimated from the data of original work of Druett *et al.* (1956). The range of dosage presented in the original work was defined as $Nt \times 10^{-4}$, where N is the number of bacteria per litre air and t is exposure time in minutes. Hence, in this study the dosage was transformed into the number of organisms inhaled by taking the product of the respiratory minute volume (litre air per minute) of the host and the factor 10^4. For guinea pigs weighing 350–450 g, the respiratory minute volume is approximately $0{\cdot}1\ \mathrm{l\ min^{-1}}$ (Kleinman and Radford 1964). However, it should be noted that the dose levels listed in Table 3 are still not reflective of the actual number of viable *Y. pestis* cells delivered into the respiratory system of the host. To calculate the number of micro-organisms inhaled with precision, one would need to know the death rate of organisms in the air, as *Y. pestis* is a very labile organism when airborne (Druett *et al.* 1956). This labile nature may explain the discrepancy in the LD_{50} dose via the respiratory route as compared with the subcutaneous one (Druett *et al.* 1956).

Noticeably in both studies of Druett *et al.* (1956) and Rogers *et al.* (2007), the deaths of mice as a response to exposure to *Y. pestis* took place from the third day and reached the highest rate on the fourth day. The critical TPI for the highest mortality rate to occur can be explained by comparison with previous kinetic studies of *in vivo* microbial growth (Lathem *et al.* 2005; Sebbane *et al.* 2005). In the study by Lathem *et al.* (2005), the pathogen load increased logarithmically in the spleens and lungs of mice, and deaths occurred by 72 h after intranasal inoculation of 10^4 CFU *Y. pestis* strain CO92. Sebbane *et al.* (2005) showed that rats succumbed due to heavy proliferation of bacteria by 72 h after intradermal inoculation of 600 CFU *Y. pestis* strain 195/P. The substantial increase in deaths between the third and the fourth day is consistent with rapid bacterial colonization and high pathogen load in animal organs after 72 h post-inoculation. In view of the 2–6 days incubation period of human bubonic plague (Benenson 1970), a possibly similar pattern of bacteria proliferation in human and mice host may exist but remains to be verified.

As described by Nishiura (2007) knowledge of incubation period distributions may be used in numerous clinical, public health and ecological studies. These potential uses of improved time-dependent dose–response models which merit the understanding of incubation period are:

1. estimation of time of exposure for a point-source outbreak

2. determining whether case onset is over in the event of an outbreak

3. early projection of disease prognosis when incubation period is associated with clinical severity

4. development of mechanistic models for infection and illness

It is feasible that by using a similar approach to examine and include the potential time dependencies into dose–response models for other pathogens and exposure routes, the temporal distributions of infections and mortalities may be described more comprehensively. Furthermore, the approach of this study demonstrates that response models may be quantitatively improved by incorporating additional physical and biological factors, e.g. the age of host and the administrated dose of antibiotics.

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