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ORIGINAL ARTICLE

Dose–response model for *Burkholderia pseudomallei* (melioidosis)

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Keywords
BALB/c mice, *Burkholderia mallei, Burkholderia pseudomallei,* C57BL/6 mice, diabetic rats, dose–response models, melioidosis.

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Abstract

Aims: The objective of this study was development of a dose–response model for exposure to *Burkholderia pseudomallei* in different animal hosts and analysis of the results. The data sets with which the model was developed were taken from the open literature.

Methods and Results: All data sets were initially tested for a trend between dose and outcome using the Cochran–Armitage test. Only data showing a statistically significant trend were subjected to further analysis (fitting with parametric dose–response relationships). Dose–response relationships (exponential, beta-Poisson and log-probit) were fit to data using the method of maximum likelihood estimation.

Conclusions: Dose–response analysis of BALB/c mice, C57BL/6 mice, guinea pigs and diabetic rats showed that BALB/c mice exposed intranasally (i.n.) and guinea pigs exposed intraperitoneally (i.p.) are significantly more sensitive to *B. pseudomallei* than C57BL/6 mice exposed i.n. and diabetic rats exposed i.p.

Significance and Impact of the Study: The results confirmed the findings of a study of outbreak data that the diabetic population is more susceptible to infection with *B. pseudomallei* than the general population. The low dose prediction from best fit dose–response models can be used to draw guidelines for public health decision making processes, including consideration of sensitive subpopulations.

Introduction

Melioidosis is an infectious, life threatening disease occurring in humans and animals, caused by the bacterium, *Burkholderia pseudomallei*. It is a flagellated Gram-negative saprophyte found in soil and water in many tropical regions including Southeast Asia, northern Australia, Central and South America, Caribbean and Africa (Leakey *et al.* 1998; Liu *et al.* 2006).

Burkholderia pseudomallei and its close species relative *Burkholderia mallei* are considered potential biological weapons and are listed as category "B" biothreat agents (Jeddeloh *et al.* 2003; Schell *et al.* 2007). *Burkholderia mallei* is the causative agent of the disease glanders in animals. Glanders is one of the oldest diseases known. Horses, donkeys and mules are the natural host animals for *B. mallei*. However, infection can occur in other animal species including camels, goats and felines. Human infection with glanders is very rare and the majority of human cases have resulted from occupational contact with infected animals (Schell *et al.* 2007).

Burkholderia pseudomallei and *B. mallei* were previously designated as *Pseudomonas pseudomallei*, *P.mallei*, *Malleomyces pseudomallei*, and *M. mallei* (Leakey *et al.* 1998; Sprague and Neubauer 2004; Anonymous 2007).

The infection of *B. pseudomallei* is acquired through skin abrasions (subcutaneous infection) inhalation or ingestion of contaminated food (soil, water) or aerosols (Barnes and Ketheesan 2005; Ulett *et al.* 2005). Clinical signs of the disease in human can vary from subacute infections to chronic suppurative (discharging pus) infections. After getting infected, a person can be in an asymptomatic state, or develop benign pneumonia, acute or chronic pneumonia and acute septicemia (Liu *et al.* 2002; Stevens *et al.* 2004).

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Compared with other bioterror agents, the study of dose response and immunity for *B. pseudomallei* is a difficult task because of the highly variable nature of the disease and wide ranges of virulence of different strains of the bacteria. There are as many as 65 strains isolated from different geographical areas and different media (Ulett *et al.* 2001; Thibault *et al.* 2004; Taweechaisupaponga *et al.* 2005; Ulett *et al.* 2005).

In this study, dose–response data from experimental infection of mice, rats and guinea pigs *to B. pseudomallei* were fit to dose–response models in an attempt to establish a human dose–response model and to explore differences in response between different species and between subpopulations among the same species with differing sensitivities. Results from this analysis may be used in the formulation of risk-based surveillance programs or public health measures that protect the population at large and sensitive subgroups.

Data and methods

Many investigators have studied the response of animals to different doses of *B. pseudomallei* and *B. mallei* bacteria in order to develop effective vaccines and to study the pathology of infected animals. Many studies have reported the lethal dose (LD_{50}) that kills (infected) 50% of the animals; fewer studies published the data sets on which their estimates were based.

Leakey *et al.* (1998) inoculated five groups of five BALB/c mice intravenously with 850, 85, 8 and 1 colony forming unit (CFU) *of B.pseudomallei*. Similarly, four groups of five C57BL/6 mice were inoculated intravenously with $1{\cdot}3 \times 10^5$, $1{\cdot}3 \times 10^4$, $1{\cdot}3 \times 10^3$ and 130 of *B. pseudomallei*. The authors reported the responses (death) and bacterial loads in vital organs of the animal (Leakey *et al.* 1998). In a similar experiment, Jeddeloh *et al.* (2003) exposed mice to aerosols of *B. pseudomallei* for future evaluation of biodefense vaccine candidates. Ulett *et al.* (2005) developed a model of immunity to *B. pseudomallei* inoculating intravenously different doses of different bacterial strains to BALB/c mice (Ulett *et al.* 2005).

Brett *et al.* (1997) administered different doses of *B. pseudomallei* to 6–8 weeks old Syrian golden hamsters via the intraperitoneal (i.p.) route. The dose range was 10 CFU to 10×10^7 CFU. Based on responses, the LD_{50} for different strains were determined (Brett, DeShazer et al. 1997). Ling *et al.* also studied the hamster model to identify virulence gene (Ling, Moore et al. 2006).

Similarly, many investigators have studied pathogenecity of *B. mallei*. Fritz *et al.* (2000) inoculated *B. mallei* i.p. to BALB/c and C57BL/6 mice. Histopathologic changes were observed and established that mice are viable models of sublethal and lethal glanders. Unfortunately, the authors did not publish dose–response data (Fritz *et al.* 2000). A study was conducted by Lever *et al.* (2003), in which BALB/c mice were infected with different doses via inhalation.

A search of the open literature identified three usable dose–response studies of *B.pseudomallei* and one of *B. mallei*. Liu *et al.* studied infection through the intranasal (i.n.) route in a murine model to mimic infection through inhalation. Two strains of mice – BALB/c and C57BL/6 – were used to explore intraspecies differences in dose–response characteristics to *B. pseudomallei*. Five doses of bacteria were given to 5–6 week old female BALB/c and C57BL/6 mice. The virulent strain KHW of *B. pseudomallei* used in the study was isolated from a person who died from melioidosis in Singapore. The bacteria were cultured on trypticase soy agar (TSA) (Liu *et al.* 2002). BALB/c and C57BL/6 mice were found to be suitable animal models for the different forms of human melioidosis; according to Leakey et al. (1998), the course of infection in BALB/c was similar to acute melioidosis in human and the C57BL/6 was appeared to be chronic melioidosis (Leakey *et al.* 1998).

Miller *et al.* (1947) explored the virulence of several strains of *B. pseudomallei* and *B. mallei* and their infectivity in several laboratory animals. Six doses of *B. pseudomallei* (strain W294) were injected i.p. to guinea pigs. Similarly, six doses of *B. mallei* (strain C7) were i.p. inoculated in guinea pigs. Mortality as the end point of response was used in both cases (Miller *et al.* 1947).

In another study carried out by Brett et al. (1996), groups of 10 diabetic rats were inoculated i.p. with *B. pseudomallei* (strain 316c) ranging from 3×10^3 CFU to 3×10^7 CFU. Sprauge–Dawley rats weighing approximately 30 g were made diabetic by streptozotocin administration. Mortality rate of diabetic rats were recorded (Brett and Woods 1996). This study is particularly important given that epidemic data of melioidosis from different parts of the world show that a significant portion of the population are diabetic (Currie et al. 2000a) and that diabetics are especially susceptible to melioidosis (Woods 1995).

Schell *et al.* (2007) studied the virulence of *B. mallei* in hamsters. Groups of five female Syrian hamsters were infected i.p. by 16 different strains of *B. mallei* and responses as death of animal were recorded (Schell *et al.* 2007).

Analysis method

All data sets were initially tested for a trend between dose and outcome using the Cochran–Armitage test (Neuhauser and Hothorn 1999). Only data showing a statistically

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significant trend were subjected to further analysis (fitting with parametric dose–response relationships). Dose–response relationships were fit to data using the method of maximum likelihood estimation (MLE) as described in Haas *et al.* (1999).

All the data sets are shown in Table 1. The statistical programming language, 'R' (http://www.r-project.org) was used for this computation. Three dose–response models (exponential, Beta-Poisson and Log-probit) were used (Haas *et al.* 1999). Exponential and Beta-Poisson MLE estimates were made using the BFGS algorithm and Log-probit estimates were made using Nelder–Mead algorithm. Confidence intervals best-fit models were determined via bootstrapping with 10 000 bootstrap iterations.

The exponential dose–response model is given by the equation 1

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

where $P(d)$ is the probability of response at dose d and k is the probability that a single organism can survive and initiate infection.

The Beta-Poisson model is given by equation (2)

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

where N_{50} is the median infective dose and α is the slope parameter for Beta-Poisson model.

The Log-probit model is given by equation (3)

Table 1 Data used in the study

Bacteria and strain	Study	Mode of inoculation	Test animal	Dose (CFU)	Number of test animals	Positive responses	Negative responses
B. pseudomallei (KHW)	Liu *et al.* (2002)	i.n.	BALB/c mice	405	6	6	0
				135	6	4	2
				45	4	3	1
				15	6	0	6
				5	6	0	6
B. pseudomallei (KHW)	Liu *et al.* (2002)	i.n.	C57BL/6 mice	12 150	6	3	3
				4050	6	3	3
				1350	6	1	5
				450	6	1	5
				150	6	0	0
*B. mallei** SR1	Schell *et al.* (2007)	i.p.	Syrian hamster	10 000	5	5	0
				1000	5	5	0
				100	5	5	0
				10	5	4	1
*B. mallei** $tssE^-/tssE^+$	Schell *et al.* (2007)	i.p.	Syrian hamster	10 000	5	5	0
				1000	5	5	0
				100	5	5	0
				10	5	3	2
B. pseudomallei W294	Miller *et al.* (1947)	i.p.	Guinea pigs	4400 000	5	4	1
				440 000	5	5	0
				44 000	5	5	0
				4400	5	4	1
				440	5	3	2
				44	5	1	4
*B. mallei** C7	Miller *et al.* (1947)	i.p.	Guinea pigs	2600 000	4	3	1
				260 000	4	3	1
				26000	4	2	2
				2600	4	3	1
				260	4	3	1
				26	4	2	2
B. pseudomallei 316c	Brett and Woods (1996)	i.p.	Diabetic rats	30 000 000	10	10	0
				3000 000	10	10	0
				300 000	10	7	3
				30 000	10	7	3
				3000	10	6	4

i.n., intranasal; i.p., intraperitoneal.
*Failed test of trend.

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

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

Goodness of fit for all models was determined by comparing the optimal value of the deviance with the critical χ^2 value at degrees of freedom equal to the number of doses minus the number of fitted parameters and a 95% confidence. Assessment of the statistical significance of improvement of fit that two parameter models provide over one parameter models was made by comparing the reduction in minimized deviance with the critical χ^2 value at one degree of freedom. Confidence intervals for the best-fit model were estimated via bootstrapping (Haas *et al.* 1999). For the bootstrapping, at least 1000 replicates were used for simulation.

Pooling analysis was performed for the different animals and bacterial species to ascertain whether the data set had the same underlying distributions. A likelihood ratio test was used to determine if data could be pooled.

Results

Cochran–Armitage test of trend

The Cochran–Armitage test showed the existence of a dose-dependent response for i.n. exposure of BALB/c and C57BAL/6 mice to *B. pseudomallei*. The Z critical values (Z_{ca}) were 4·14 and 2·33 respectively and the values were greater than 1·64 which was above the upper fifth percentile of the normal distribution.

Dose–response data for *B. pseudomallei* inoculated i.p. in guinea pigs also showed a positive trend. The Z_{ca} value was 2·36. Similarly, data of diabetic rats inoculated with *B. pseduomallei* had a positive trend (Z_{ca} as 2·75). Hence, the null hypothesis of lack of trend is rejected in those cases.

On the other hand, *B. mallei* exposed to guinea pigs (Z_{ca} was 1·01) and both the data set of *B. mallei* exposed to Syrian hamsters (Z_{ca} were 1·26 and −3·65) showed lack of trend (Z_{ca} value of 1·64). As the result, a quantitative dose–response analysis of those data is not justified.

Dose–response of BALB/c mice

The best-fit dose response model for mortality in i.n. inoculated BALB/c mice was the exponential model. Although the two parameter models had smaller minimized deviances, the improvement in fit provided by those models was not statistically significant. Statistics of the three model fits to the animals are summarized in Table 2 and best fit model with confidence interval is shown in Fig. 1.

Dose–response of C57BL/6 mice

The best fit dose–response model for mortality in i.n. inoculated C57BL/6 mice was again the exponential model. Statistics of the three model fits to the animals are summarized in Table 3 and best fit model with confidence interval is shown in Fig. 2.

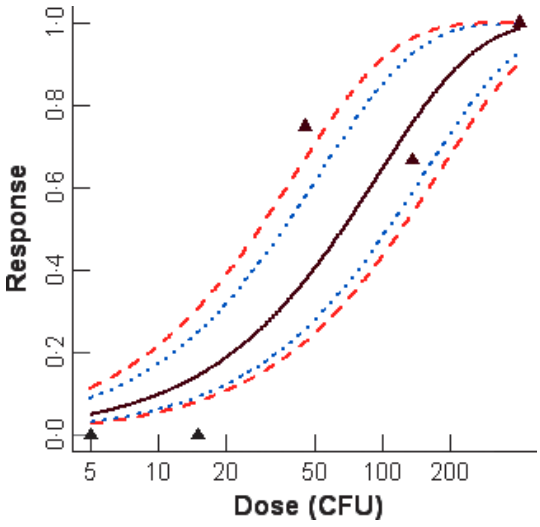

Figure 1 Dose–response data and exponential model fits for mortality in BALB/c mice exposed i.n. to *B. pseudomallei*. (▬▬) Best fit model, (▬ ▬) 95% confidence, (▪▪▪▪) 99% confidence, (▲) data.

Table 2 Model fit comparison for mortality in intranasal exposed BALB/c mice (Liu *et al.* 2002)

Data set	Number of doses	Model	Minimized deviance	Degrees of freedom	Parameters
Burkholderia pseudomallei	5	Exponential*	5·250	4	k = 0·010448
BALB/c	5	Beta Poisson	5·250	3	α = 1·0062e7
					N_{50} = 66·339
	5	Log-probit	4·077	3	q_1 = 0·932
					q_2 = 58·024

*Accepted best fit model.

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Table 3 Model fit comparison for mortality in intranasal exposed C57BL/6 mice (Liu *et al.* 2002)

Data set	Number of doses	Model	Minimized deviance	Degrees of freedom	Parameters
Burkholderia pseudomallei	5	Exponential*	3·345	4	k = 0·0001004336
C57BL/6	5	Beta-Poisson	1·192	3	α = 0·317 N_{50} = 8204·71
	5	Log-probit	1·284	3	q_1 = 2·271 q_2 = 7831·219

*Accepted best fit model.

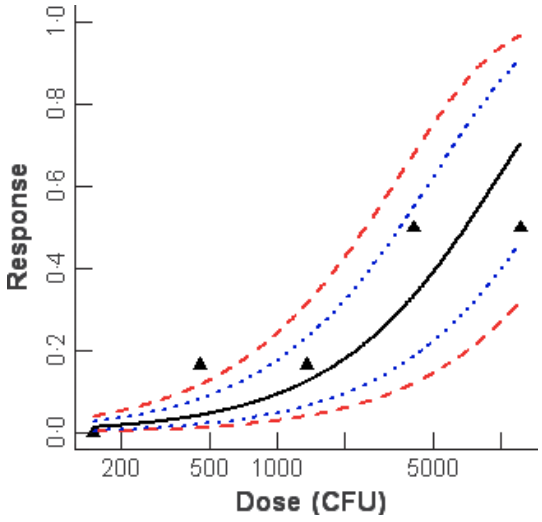

Figure 2 Dose–response data and exponential model fits for mortality in C57BL/6 mice i.n. exposed to *B. pseudomallei*. (▬▬) Best fit model, (▬ ▬) 95% confidence, (▪ ▪ ▪ ▪) 99% confidence, (▲) data.

Leakey *et al.* have demonstrated that BALB/c mice were more susceptible to *B. pseudomallei* than C57BL/c mice. An experiment on BALB/c and C57BL/6 mice with various doses of *B.pseudomallei* showed that contrasting susceptibility levels in those mouse strains. Rapidly increasing bacteremia in BALB/c mice and absence of variable bacteria in the blood of C57BL/6 mice after 96 h of inoculation showed the contrasting susceptibility (Leakey *et al.* 1998). BALB/c mice died due to septicemic disease with towering bacterial loads in organs such as liver, spleen, lungs and blood, supplemented by organ inflammation and necrosis a few days after infection. It reflects a failure of the host innate immune response to contain the infection. In the organs of infected BALB/c mice, early infiltration and accumulation of neutrophils was evident, whereas in C57BL/6 mice, macrophage accumulation was more evident. Hence, the better control of infection in C57BL/6 mice is possibly due to macrophages. Moreover, the resistance mechanisms are inadequate in BALB/c mice, resulting in bacterial growth and inflammation (Ulett *et al.* 1998; Gan 2005).

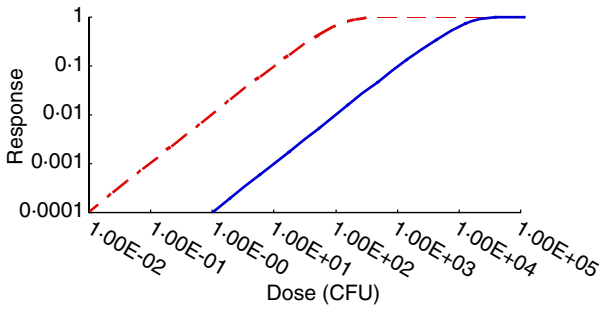

Figure 3 Low dose prediction and comparative dose–response of BALB/c and C57BL/6 mice exposed to *B. pseudomallei* (i.n. exposure). (▬ · ▬) C57 BL/6 mice, (▬▬) BALB/c mice. Exponential model was the best fit model.

The low dose predictions and comparative virulence is given in Fig. 3.

Dose–response of guinea pigs

The best fit dose–response model for mortality in i.p. inoculated guinea pigs was the Beta-Poisson model. Statistics of the three model fits to the animal is summarized in Table 4 and the best fit model and confidence interval are shown in Fig. 4.

Dose–response of diabetic rats

For diabetic rats exposed to *B. pseudomallei*, both the Beta-Poisson and Log-probit models provided statistically acceptable fits. However, the Beta-Poisson model was preferred to Log-probit. The Beta-Poisson model has been used to describe dose–response of many other infectious agents. Second, the Beta-Poisson can be derived from mechanistic principles describing the action of infectious agents, while the Log-probit is nonmechanistic and purely empirical. The Beta-Poisson model describes the host dose–response relationships to micro-organism and it takes into account the variation that exist in pathogen host interactions (Haas 1983; Haas *et al.* 2000). Statistics of the fits of the dose–response models are shown in Table 5 and the best fit model with confidence intervals is

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Table 4 Model fit comparison for mortality in peritoneally exposed guinea pigs (Miller *et al.* 1947)

Data set	Number of doses	Model	Minimized deviance	Degrees of freedom	Parameters
Burkholderia pseudomallei	6	Exponential	70·72928	5	k = 0·0007450
KHW	6	Beta-Poisson*	3·973655	4	α = 0·25060 N_{50} = 192·264
	6	Log-probit	5·167541	4	q_1 = 5·990 q_2 = 116·044

*Accepted best fit model.

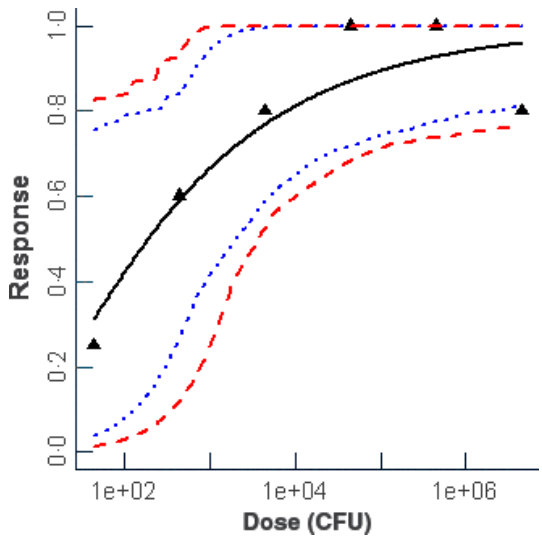

Figure 4 Dose–response data and Beta-Poisson model fits for mortality in peritoneally exposed guinea pigs to *B. pseudomallei*. (▬▬) Best fit model, (▬ ▬) 95% confidence, (▪ ▪ ▪ ▪) 99% donfidence, (▲) data.

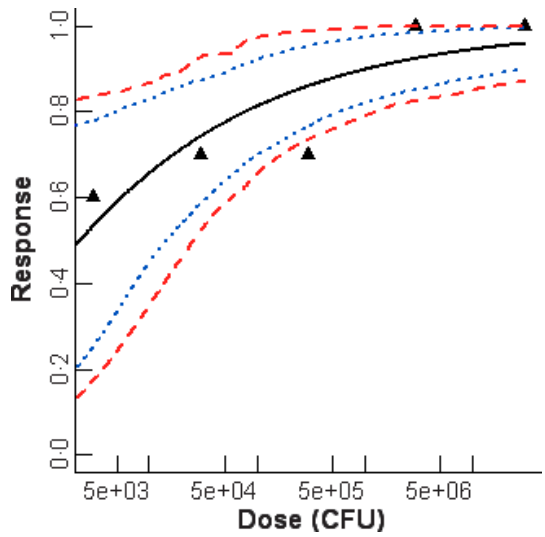

Figure 5 Dose–response data and Beta-Poisson model fits for mortality in peritoneally exposed diabetic rats to *B. pseudomallei*. (▬▬) Best fit model, (▬ ▬) 95% confidence, (▪ ▪ ▪ ▪) 99% confidence, (▲) data.

shown in Fig. 5. At very low doses (well below the observed range), the Log-probit model predicts higher responses than the Beta-Poisson model. The comparative low dose predictions are shown in Fig. 6. However the two curves deviate well below the observed range, and so targeted studies at very low dose would be required to differentiate between these alternative models.

Pooling analysis

The data for the two strains of mice (BALB/c and C57BL/6) exposed i.n. to *B. pseudomallei* could not be pooled indicating that they were not from same distribution, although the routes of infection were the same. But the pooling was possible in data sets from different animals and different strains of bacteria. The data for C57BL/6 mice exposed i.n. to *B. pseudomallei* could be pooled with the data for diabetic rats exposed i.p. to *B. pseudomallei* (Fig. 7). Similarly, the data for guinea pigs 60 exposed i.p. to *B. pseudomallei* could be pooled with the data for diabetic rats exposed i.p. to *B. pseudomallei* (Fig. 8).

Table 5 Model fit comparison for mortality in peritoneally exposed diabetic rats (Brett and Woods 1996)

Data set	Number of doses	Model	Minimized deviance	Degrees of freedom	Parameters
Burkholderia pseudomallei	5	Exponential	43·38476	4	k = 1·231171e^{-05}
Diabetic rats	5	Beta-Poisson*	4·392305	3	α = 0·2649 N_{50} = 2273·10
	5	Log-probit	3·435624	3	q_1 = 4·628007 q_2 = 2293·720

*Accepted best fit model.

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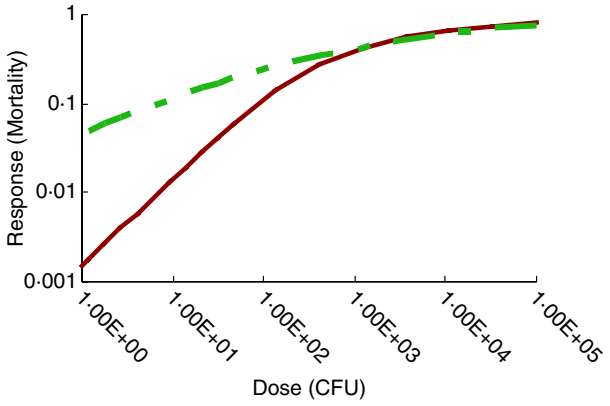

Figure 6 Low dose predictions for Log-probit and Beta-Poisson models for diabetic rat exposed to *B. pseudomallei*. (▬ · ▬) Beta-Poisson, (▬▬) Log-probit.

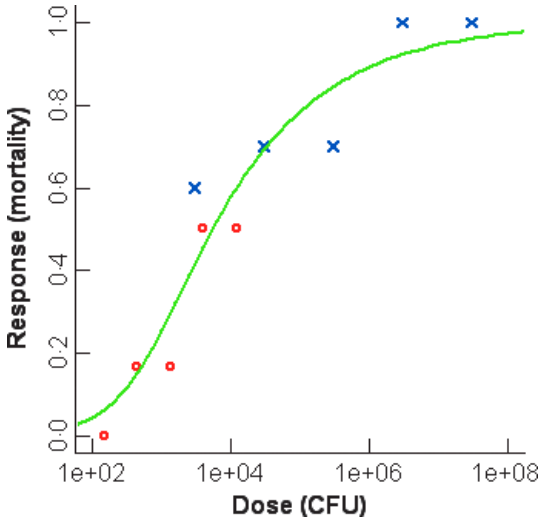

Figure 7 Combined set of dose–response points of C57BL/6 mice exposed i.n. and diabetic rats exposed i.p. to *B. pseudomallei*, and pooled best fit model (Beta-Poisson). (x) C57BL/6 mice, (o) diabetic rats, (▬▬) pooled data.

The comparative study of virulence in different hosts

The comparative low dose predictions for C57BL/6 mice model and Diabetic rat model are shown in Fig. 9. The comparative virulence *B. pseudomallei* in different hosts (BALB/c mice, C57BL/6 mice and Diabetic rats) were summarized in Fig.10. The virulence of bacteria in the host guinea pigs was almost similar to BALB/c mice as shown in Fig. 10.

Discussion

Different strains of *B. pseudomallei* are found to vary widely in virulence in the same animal host and the same

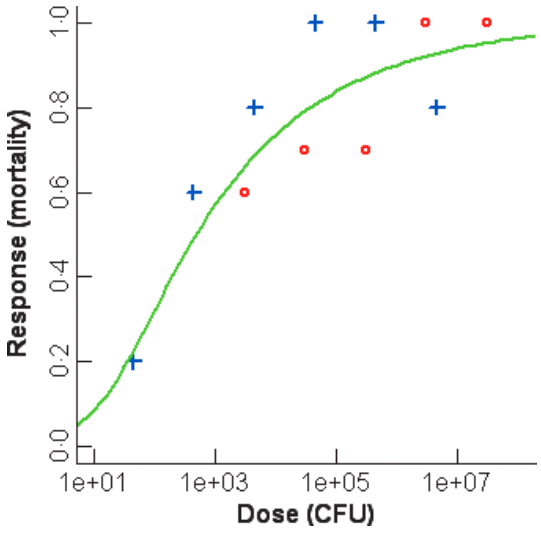

Figure 8 Combined set of dose–response points of diabetic rats and guinea pigs exposed i.p. to *B. pseudomallei*, and pooled best fit model (Beta-Poisson). (+) Guinea pigs, (o) diabetic rats, (▬▬) pooled data.

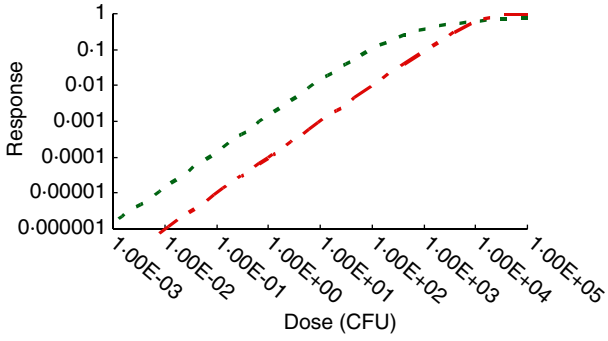

Figure 9 Dose prediction for diabetic rats and C57BL/6 mice model exposed to *B. pseudomallei*. (▪ ▪ ▪ ▪) Diabetic rats- Beta-Poisson model, (▬ · ▬).

strain of *B. pseudomallei* can have very different virulence depending on the animal host. The reported LD_{50} for the intravenous route of exposure is in the range of 3 CFU to $4{\cdot}43 \times 10^6$ CFU in BALB/c mice (Ulett *et al.* 2001) Some of the virulence in different animals are shown in Table 6. Both the strains of mice showed similar responses in term of dose–response model as both of them resulted in Exponential dose–response. However, the virulence of the bacteria in those two host strains was markedly different. The data for *B. pseudomallei* in two strains of mice (BALB/c and C57BL/6) could not be pooled supporting the fact that they were not from same distribution and having different sensitivity factors. The biological reasons were unknown but the reason behind it might be huge differences in susceptibility of infection in these two strains. In i.p. inoculated guinea pigs, the response was

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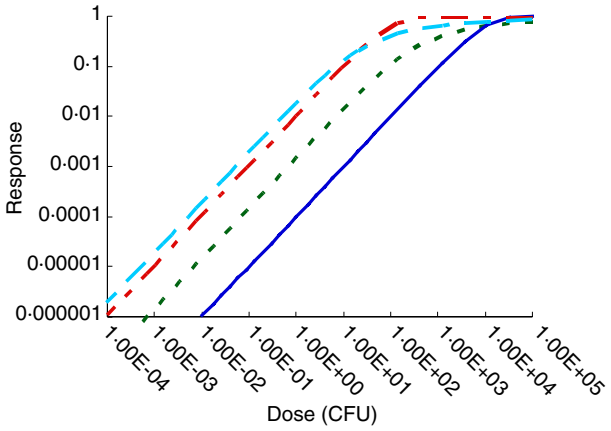

Figure 10 Comparative low dose prediction of *B. pseudomallei* for BALB/c mice, C57BL/6 mice, diabetic rats and guinea pigs. (▬ ▬) Guinea pigs, (▬ · ▬) BALB/c mice, (▪ ▪ ▪ ▪) diabetic rats, (▬▬) C57Bl/6 mice.

best fit by the Beta-Poisson distribution. In contrast to above models, the diabetic rats inoculated with *B. pseudomallei* i.p. route showed both Log-probit and Beta-Poisson responses. However, as discussed earlier, Beta-Poisson was preferred to Log-probit.

Many studies have shown that a higher risk of melioidosis is associated with diabetes mellitus. Woods *et al.* (1999) demonstrated that insulin markedly inhibits the growth of *B. pseudomallei*. A significant percentage of melioidosis patients have been found to be diabetic ranging from 36% of total infected populations (Currie *et al.* 2000b) to 60% (Suputtamongkol *et al.* 1999). One of the reasons for the association between diabetes and melioidosis is that the innate immunity of diabetic person is suppressed especially the neutrophil function (Gan 2005). The figure is well supported by the analysis of outbreak of Melioidosis in Northern Australia by Merianos *et al.* (1993). This outbreak in Northern Territory of Australia from 1 November 1990 to 30 June 1991 showed that the individual attack rate of melioidosis was $3{\cdot}42 \times 10^{-4}$ per year and that of diabetic population was $2{\cdot}283 \times 10^{-3}$, indicating that diabetic population is 6·67 times more susceptible than general population.

Interestingly, although the routes of infection are different, the data of *B. pseudomallei* exposed to C57BL/6 mice (i.n.) and Diabetic rats (i.p.) can be pooled as shown in Fig. 7. The pooling simply implies that they may be from the same distribution and the predicted responses will be the same regardless the animals and routes of infection. However the experimental results and dose–response analysis showed markedly different responses. Dannenberg et al. (1958) studied that pathogenecity of *B. pseudomallei* in different hosts and different routes of the infection. The experimental results showed

Table 6 Hosts, routes of infection and virulence

	Host			
	BALB/c mice	C57BL/6 mice	Guinea pigs	Diabetic rats
Routes of Infection				
i.p.	LD_{50} = 12 to 4·7 × 10^5 (CFU) (Barnes and Ketheesan 2005)	LD_{50} = 9·73 × 10^5 to 2·1 × 10^7 (CFU)* (Barnes and Ketheesan 2005)	LD_{50} = 4854 (CFU) best fit model = Beta-Poisson α = 0·250, N_{50} = 192·264 (Miller, Pannel *et al.* 1947)	LD_{50} = 1·96 (CFU), best fit model = Beta-Poisson, α = 0·265, N_{52} = 273·10* (Brett and Woods 1996)
i.n.	LD_{50} = 45 (CFU), best fit model = exponetial, k = 0·010 (Liu *et al.* 2002)	LD_{50} = 4854 (CFU), best fit model= exponential k = 0·010 * (Liu *et al.* 2002)		
i.v.	LD_{50} = <10 to 9 × 10^3 (CFU) (Barnes and Ketheesan 2005)	LD_{50} = 5 × 10^3 to 6 × 10^6 (CFU) (Barnes and Ketheesan 2005)		
s.c.	LD_{50} = 1000 to 9 × 10^2 (CFU)	LD_{50} = 900 to >10^8 (CFU) (Barnes and Ketheesan 2005)		
Oral	LD_{50} = 7·2 × 10^3 to 4·8 × 10^6 (CFU) (Barnes and Ketheesan 2005)	LD_{50} = 1·8 × 10^6 to >10^8 (CFU) (Barnes and Ketheesan 2005)		

*Data can be pooled

i.p., intraperitoneal; i.n., intranasal; i.v., intravenous; s.c., subcutaneous.

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that there were significant bacterial loads in lungs in both routes of inoculation. In case of respiratory route, the average bacterial titres 2 h after inoculation were $9{\cdot}5 \times 10^4$ in the lung of mice when inoculum dose was $1{\cdot}6 \times 10^6$ to $1{\cdot}7 \times 10^7$ organisms. In the case of i.p. route of inoculation, the titres 3–4 h after injection were $2{\cdot}3 \times 10^6$ when inoculum dose was $1{\cdot}1 \times 10^8$ organisms (Dannenberg and Scott 1958a). In both cases, pulmonary lesions developed swarmed with bacteria and large abscesses were present in lungs (Dannenberg and Scott 1958b). This may be case of acute pulmonary form of disease characterized by high fever and pulmonary distress which is followed by visceral abscesses and death within few days (Brett and Woods 2000). Another explanations behind the possible pooling of the two different routes of infection may be the motility of the organism (Brett and Woods 2000). The flagellated bacteria are too motile to reach the target organs regardless the routes of infection before the host immune system to act.

However, the comparative virulence, shown in Fig. 10, clearly indicates that diabetic rats are more susceptible to *B .pseudomallei* than C57BL/6 mice at lower doses less than 10 000 CFU. At 10^4 CFU, the responses were almost same (0·63 and 0·65), where as at 1 CFU, the response in diabetic rats were 15 times that of C57BL/c mice. Similarly at 100 CFU, responses in diabetic rats were 11 times higher than that of mice. These figures are consistent with the results of outbreak data analysis of outbreak of melioidosis in Northern territory of Australia.

One means of comparing the risks in two strains of mice and diabetic rat infection from *B.pseudomallei*, as shown in Fig. 10, is to compute the doses which result in a risk of 1/1000; the corresponding doses for BALB/c mice, C57BL/6 mice and diabetic rat are 0·1, 10 and 1·0 organisms respectively. In other words, BALB/c mice need 10 times less doses of *B. pseudomallei* (exposed i.n.) than diabetic rats (exposed i.p.) and C57BL/6 mice need 10 times more doses (exposed i.n.) to have risk of 1/1000. The observations are drawn regardless the routes of infection. Barnes and Ketheesan (2005) demonstrated that different routes of infection had different virulence in the hosts. The experiments showed that LD_{50} of i.p. infection was almost 10 times less than i.n. infection in mice (Barnes and Ketheesan 2005).

The response of guinea pig to *B. pseudomallei* is almost similar to response of BALB/c mice and highly susceptible at low and high doses.

Similarly, the data set of *B. pseudomallei* exposed to guinea pigs and diabetic rats both of them inoculated i.p., can also be pooled (shown in Fig. 8), despite different hosts, indicating the animals belong to the same distribution. But the differences in susceptibility tell another side of story that they are not.

Conclusion

Based on differences in dose–response models fit to data for BALB/c mice, C57BL/6 mice, guinea pigs and diabetic rats, BALB/c mice (exposed i.n.) and guinea pigs (exposed i.p.) are significantly more sensitive to *B. pseudomallei* than C57BL/6 mice (exposed i.n.) and diabetic rats (exposed i.p.). Because of lack of data availability in open literature, the virulence of same strain of *B. pseudomallei* to different hosts could not be compared. However, in spite of pooling of result suggests that the dose–responses may be same in both cases, the individual results from dose–response analysis and outbreak data analysis support the fact that diabetic population is more susceptible than general people. But we were unable to compare the dose–response in nondiabetic and diabetic animals for the same strains of bacteria. This study demonstrates the need for experiments in which greater numbers of animals are exposed to different strains of bacteria and for experiments conducted with all the relevant exposure routes.

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