

ORIGINAL ARTICLE

# Dose–response time modelling for highly pathogenic avian influenza A (H5N1) virus infection

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disease(s), infection, modelling, virulence, virus(es).

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## Abstract

**Aims:** To develop time-dependent dose–response models for highly pathogenic avian influenza A (HPAI) of the H5N1 subtype virus.

**Methods and Results:** A total of four candidate time-dependent dose–response models were fitted to four survival data sets for animals (mice or ferrets) exposed to graded doses of HPAI H5N1 virus using the maximum-likelihood estimation. A beta-Poisson dose–response model with the  $N_{50}$  parameter modified by an exponential-inverse-power time dependency or an exponential dose–response model with the  $k$  parameter modified by an exponential-inverse time dependency provided a statistically adequate fit to the observed survival data.

**Conclusions:** We have successfully developed the time-dependent dose–response models to describe the mortality of animals exposed to an HPAI H5N1 virus. The developed model describes the mortality over time and represents observed experimental responses accurately.

**Significance and Impact of the Study:** This is the first study describing time-dependent dose–response models for HPAI H5N1 virus. The developed models will be a useful tool for estimating the mortality of HPAI H5N1 virus, which may depend on time postexposure, for the preparation of a future influenza pandemic caused by this lethal virus.

## Introduction

Influenza A viruses are members of the family *Orthomyxoviridae*, which comprises enveloped viruses with segmented, negative-sense RNA genomes (Wright *et al.* 2007). Based on the antigenicity of the two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), influenza A viruses are currently divided into 16 HA and 9 NA subtypes, designated as H1–H16 and N1–N9. Over the past century, only viruses of the H1N1, H2N2, H3N2 and H1N2 subtypes have circulated in humans (Wright *et al.* 2007). Highly pathogenic avian influenza A (HPAI) of the H5N1 subtype viruses cause severe disease in humans, characterized by rapidly progressive pneumonia, multiorgan dysfunction and high mortality rate of more

than 50% (Neumann *et al.* 2010). Since 2003, they have spread across large parts of the globe and continued to cause sporadic human infections. Although HPAI H5N1 viruses fortunately have not acquired the ability for efficient infection in and transmission between humans, statistical evidence of human-to-human transmission has been obtained from epidemiological studies (Yang *et al.* 2007), suggesting the potential to acquire the ability of sustained human-to-human transmission. To prepare for the worst (namely, the occurrence of an influenza pandemic by this lethal virus), it is essential to evaluate the infectivity and pathogenicity of HPAI H5N1 virus.

Quantitative microbial risk assessment (QMRA) framework can be a powerful tool to understand how to control pandemics mediated by environmental reservoirs or

human-to-human transmission (e.g. calculating the risk of infection because of a low dose). An essential step in the QMRA process is dose-response assessment (Haas *et al.* 1999). Dose-response relationships have been investigated for many types of pathogens, e.g. *Escherichia coli* O157:H7, rotavirus, *Cryptosporidium parvum* (Haas *et al.* 1993, 2000; Teunis *et al.* 2002), by predicting the response from exposure to a given dose. Recently, the time-dependent dose-response models have been developed for improving postexposure decision-making and/or for estimating exposure time for a point-source outbreak, and they were proposed as an advanced approach for future QMRA frameworks (Huang and Haas 2009a; Huang *et al.* 2009b). As the mortality of humans infected with HPAI H5N1 virus is dependent on the days postexposure, based on the clinical observations (Liem *et al.* 2009), it is desirable to utilize time-dependent dose-response model for assessing and characterizing the risks of HPAI H5N1 virus infection to humans. However, to date, none of the prior studies investigated the dose-response relationship to describe HPAI H5N1 virus infection.

This study was performed on the basis of the above-mentioned background to develop time-dependent dose-response models for HPAI H5N1 virus to describe mortality that depends on time postexposure.

## Methods

### Source of data

There are no data sets challenging humans with wild-type HPAI H5N1 virus, because of its extreme high pathogenesis and mortality. Therefore, we used the alternative data sets describing pathogenesis of the virus to mice and ferrets as mammalian models (data set nos. 1–4), as described in Table 1. Briefly, the animals were intranasally or intratracheally inoculated with wild-type HPAI H5N1 virus, and the time-dependent response data were then obtained by monitoring the mortality of the animals (Fan *et al.* 2009; van den Brand *et al.* 2010; Wang *et al.* 2010; Kiso *et al.* 2011). These data sets include different experimental conditions of hosts, virus strains and inoculation routes.

### Candidate dose-response models based on time dependency

It is well known that the exponential and the beta-Poisson models provide good fits for microbial dose-response data, and these models have been used widely for risk assessment (Haas *et al.* 1999). The equations of exponential and beta-Poisson models are shown as Eqns (1) and (2), respectively.

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

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

where  $P(d)$  represents the probability of infection at the dose of  $d$ ,  $k, N_{50}, \alpha$  are parameters specific for the pair of host and pathogen.

As survival of animals exposed to HPAI H5N1 virus was dependent on days postinoculation (DPI) (Fan *et al.* 2009; van den Brand *et al.* 2010; Wang *et al.* 2010; Kiso *et al.* 2011), the classical dose-response models were expanded to incorporate DPI dependency by including additional parameters. The classical exponential and beta-Poisson models were expanded to include exponential-inverse or exponential-inverse-power time dependencies into  $k$  and  $N_{50}$  values, respectively, and they were assumed as candidate time-dependent models (Table 2).

### Estimation of the parameters of time-dependent dose-response models

The candidate models were then applied for parameter estimation, as described previously (Huang and Haas 2009a; Huang *et al.* 2009b). Briefly, maximum-likelihood estimation (MLE) method (Haas *et al.* 1999) implemented into the R programming language (<http://www.r-project.org>) was used to fit candidate models to observed data. For both the exponential and the beta-Poisson models, the Broyden-Fletcher-Goldfarb-Shannon algorithm was used for optimization. The goodness of fit for the two models was determined based on their likelihoods, by comparing the deviances with the critical values of the chi-squared distribution at a 95% confidence level

**Table 1** Data sets on time-dependent dose-response relationship of animals infected with highly pathogenic avian influenza A (H5N1) viruses

Data set no.	Virus strain	Host	Exposure	No. of dose point	No. of time point	No. of animals per group	Reference
1	A/duck/Guangxi/35/01	BALB/c mice	Intranasal	6	14	5	Fan <i>et al.</i> 2009
2	A/Hanoi/30408/2005	BALB/c mice	Intranasal	7	21	4	Kiso <i>et al.</i> 2011
3	A/Vietnam/1203/04	Ferrets	Intranasal	2	11	12	Wang <i>et al.</i> 2010
4	A/Indonesia/5/2005	Ferrets	Intratracheal	2	6	6	van den Brand <i>et al.</i> 2010

**Table 2** DPI-dependent dose-response model description

Model description		DPI-dependent parameter	DPI-dependent dose-response model	References
Basic model	DPI dependency			
Exponential	Exponential-inverse	$k = e^{(k_0/DPI+k_1)}$ (3a)	$P(d) = 1 - e^{-e^{(k_0/DPI+k_1)}d}$ (3b)	Huang and Haas 2009a; Huang <i>et al.</i> 2009b
	Exponential-inverse-power	$k = e^{[k_0/(DPI)^{k_2}+k_1]}$ (4a)	$P(d) = 1 - e^{-e^{[k_0/(DPI)^{k_2}+k_1]}d}$ (4b)	This study
beta-Poisson	Exponential-inverse	$N_{50} = e^{[j_0/(DPI)^{j_1}]}$ (5a)	$P(d) = 1 - [1 + \frac{d}{e^{[j_0/(DPI)^{j_1}]}} \times (2^{\frac{1}{2}} - 1)]^{-\alpha}$ (5b)	Huang and Haas 2009a; Huang <i>et al.</i> 2009b
	Exponential-inverse-power	$N_{50} = e^{[j_0/(DPI)^{j_2}+j_1]}$ (6a)	$P(d) = 1 - [1 + \frac{d}{e^{[j_0/(DPI)^{j_2}+j_1]}} \times (2^{\frac{1}{2}} - 1)]^{-\alpha}$ (6b)	This study

DPI, days postinoculation.

$\chi^2_{0.95,df}$  ( $df = m - n$ ), where  $df$  is the degree of freedom,  $m$  is the number of doses and  $n$  is the number of parameters. An acceptable fit must have a deviance lower than  $\chi^2_{0.95,df}$ . To determine a better model, the differences in deviances between two models were compared with the 95% confidence value of chi-squared distribution at a degree of freedom equal to the difference in parameter numbers between these two models ( $\chi^2_{0.95,\Delta df}$ , where  $\Delta df$  is the difference in the degrees of freedom between two models).

## Results

### Identification of best-fit model

To identify the best model to describe time-dependent dose-response relationship for HPAI H5N1 virus, the survival data listed in Table 1 were fitted to each of four candidate time-dependent models based on exponential and beta-Poisson dose-response models using MLE. The estimated parameters and the minimized deviances were determined as listed in Table 3. For data set nos. 1 and 2,

the four-parameter beta-Poisson model with exponential-inverse-power DPI dependency (Eqn 6b in Table 2) proved to be the best-fit model among the candidate models with the lowest minimized deviances and gave a statistically significant improvement in fit over the two- or three-parameter models by reducing the deviance by more than  $\chi^2_{0.95,2}$  or  $\chi^2_{0.95,1}$ , respectively (Table 3). In contrast, the two-parameter exponential with exponential-inverse DPI dependency (eqn 3b in Table 2), the simplest model among the four candidate models, was judged to be the best-fit model for data set no. 3 and 4 (Table 3).

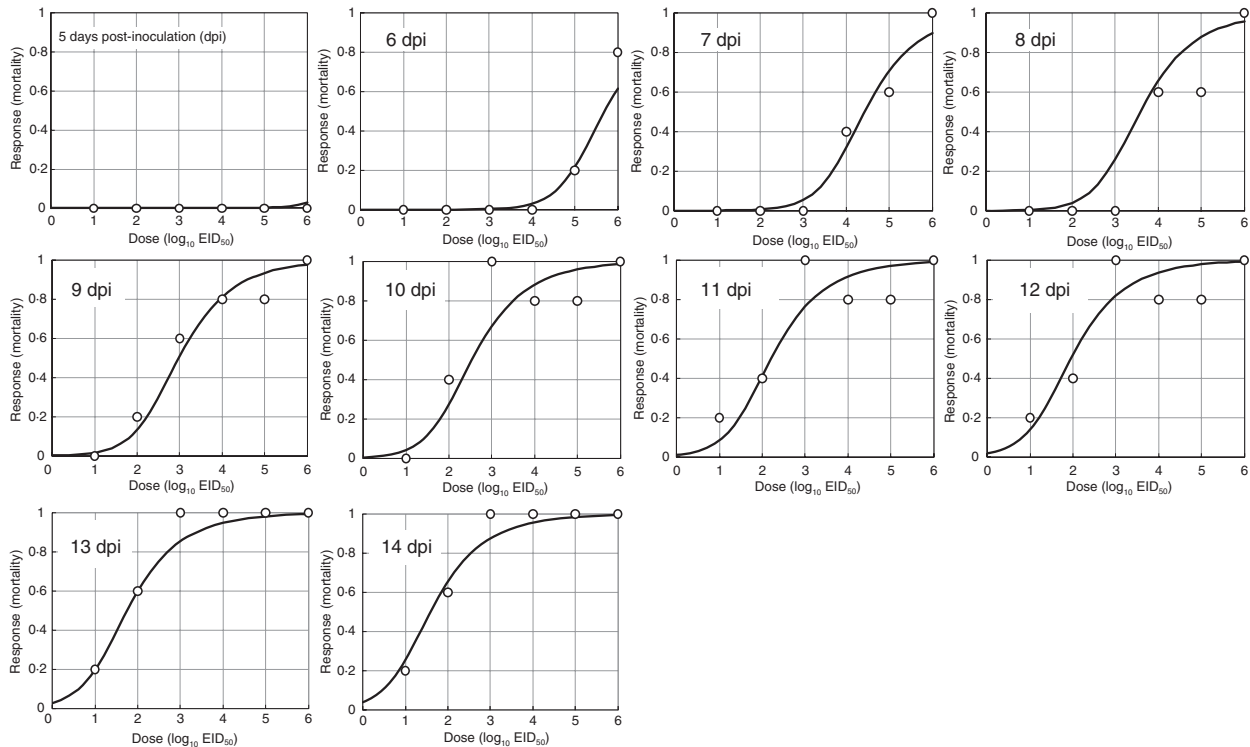
### Comparison between the best-fit model and experimental data sets

The dose-response or time-response curves of the best-fit models were compared with the observed mortalities, as shown in Figs 1–4. Although the experimental data sets include different experimental conditions of experimental animals, HPAI H5N1 virus strains and inoculation routes, the best-fit model for each data set well predicted the observed animal responses.

**Table 3** Optimal parameter estimates and minimized deviances of best-fit models

Data set no.	Best-fit model description		No. of parameters	Parameter estimates	Minimized deviance	$\chi^2_{0.95, df}$
	Basic model	DPI dependency				
1	beta-Poisson	Exponential-inverse-power	4	$\alpha = 4.640 \times 10^{-1}$ $J_0 = 3.015 \times 10^2$ $J_1 = 1.000$ $J_2 = 1.793$	34.7	101.9
2	beta-Poisson	Exponential-inverse-power	4	$\alpha = 2.730 \times 10^{-1}$ $J_0 = 9.617 \times 10^4$ $J_1 = 2.7082$ $J_2 = 4.666$	39.6	171.9
3	Exponential	Exponential-inverse	2	$k_0 = -1.707 \times 10^1$ $k_1 = -1.502 \times 10^{-1}$	15.2	31.4
4	Exponential	Exponential-inverse	2	$k_0 = -1.480 \times 10^1$ $k_1 = -7.092$	3.1	18.307

DPI, days postinoculation.



**Figure 1** The best-fit model (beta-Poisson model with exponential-inverse-power DPI dependency) (curves) compared to observed mortalities against doses (symbols) from the study of Fan *et al.* (2009) (data set no.1).

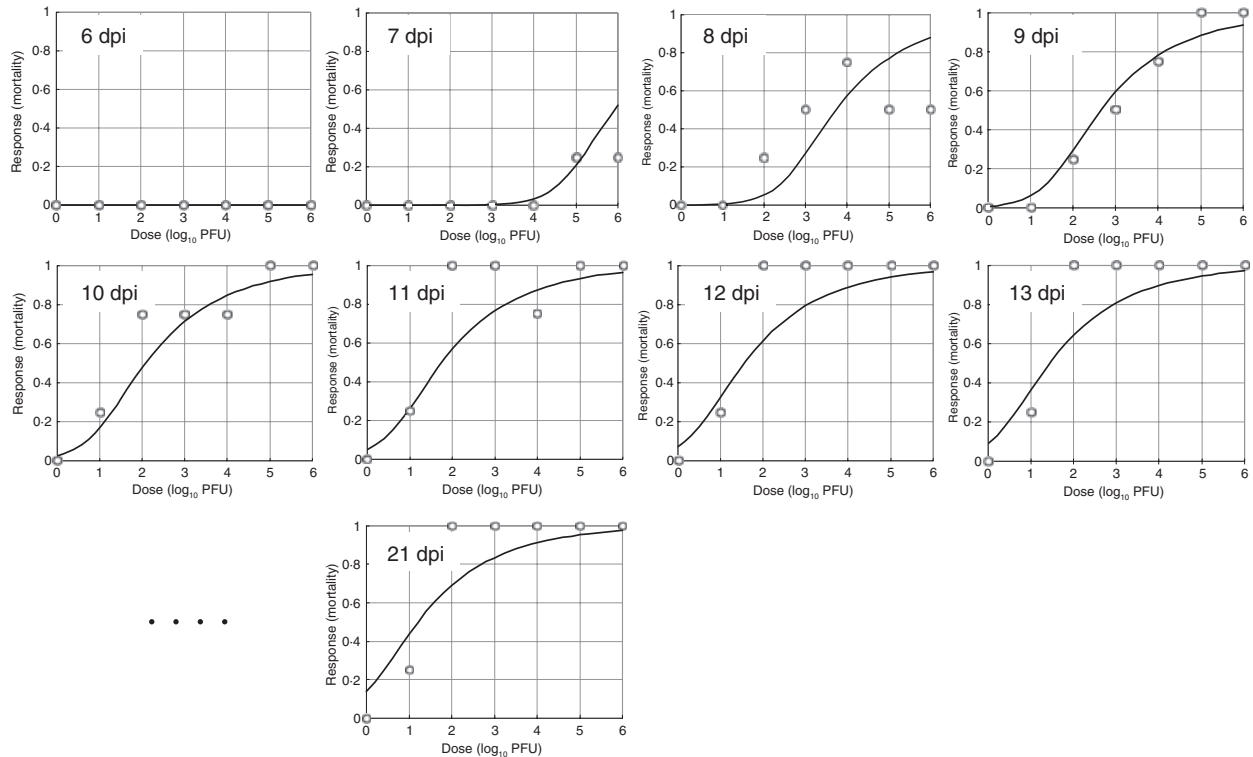
## Discussion

Although human infection with HPAI H5N1 virus is of a great public health concern, dose–response model of the virus has not been reported. This is partly because there are no data sets describing human challenge with wild-type HPAI H5N1 virus because of its high mortality. This situation seems common to other pathogens with a high virulence, such as SARS coronavirus (Watanabe *et al.* 2010). Prior microbial dose–response studies on several pathogens, however, have demonstrated that data from animal experiments provide reasonable estimates for human susceptibility (Haas *et al.* 2000; Armstrong and Haas 2007; Bartrand *et al.* 2008). Mice have also been widely used as mammalian models to study the pathogenesis of HPAI H5N1 virus; a major advantage of this model is that infection experiments can be performed with large groups of animals, because of the relatively low cost and easy husbandry, to achieve statistical significance (Belser *et al.* 2009). Katz *et al.* (2000a) reported that H5N1-infected mice exhibited inflammatory cell infiltration that is also observed for human fatal cases associated with HPAI H5N1 virus infection (de Jong 2008). Ferrets are excellent model to study the pathogenesis and transmissibility of influenza viruses because their clinical

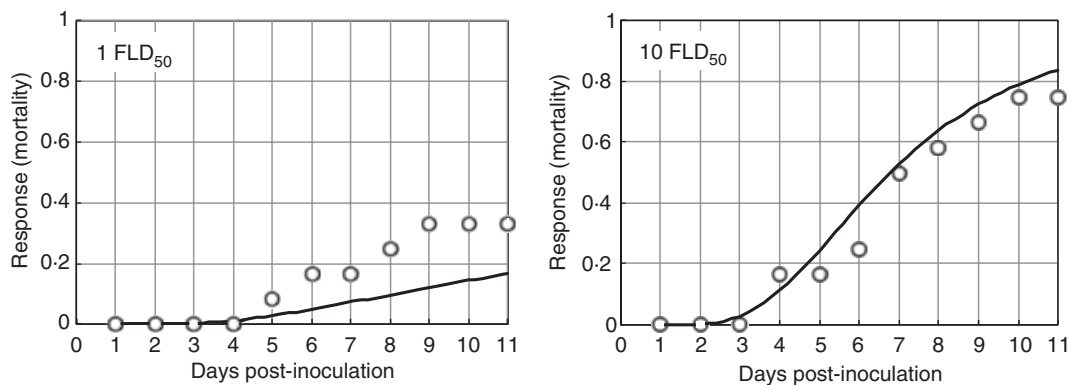
symptoms following influenza virus infection are similar to those of humans (Zitzow *et al.* 2002; Belser *et al.* 2009).

In the present study, we used the data sets including different experimental conditions of hosts, virus strains and inoculation routes and investigated the time-dependent dose–response relationship of HPAI H5N1 virus. The exponential and the beta-Poisson models, both assuming the random (i.e. Poisson) distribution of pathogens between doses (Haas *et al.* 1999), usually provide good fits for microbial dose–response data and have been used for risk assessment. In the present study, we constructed candidate time-dependent models by incorporating time factor into the exponential or the beta-Poisson model (Table 2), because it is reasonable to assume the random distribution of HPAI H5N1 virus. We found that the best-fit model differed depending on the data set (Table 3), probably due to the difference in host, virus strain and/or inoculation route.

It should be noted that 50% mice lethal dose (MLD<sub>50</sub>) values, which are the most commonly used lethality indicator of HPAI H5N1 viruses, are highly variable from  $<10^{1.5}$  to more than  $10^7$  depending on the strain (Lu *et al.* 1999; Katz *et al.* 2000b; Nguyen *et al.* 2005; Suguitan *et al.* 2006), suggesting that the lethality of HPAI H5N1 virus is highly variable. To calculate infection risk



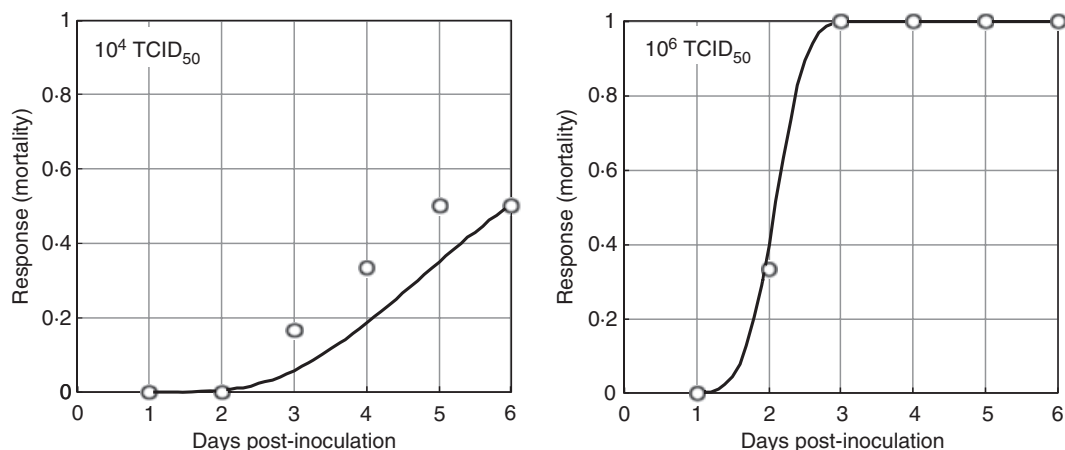
**Figure 2** The best-fit model (beta-Poisson model with exponential-inverse-power DPI dependency) (curves) compared to observed mortalities against doses (symbols) from the study of Kiso *et al.* (2011) (data set no.2). Results for 14–20 dpi were not shown since the observed mortalities did not change between 12 and 21 dpi.



**Figure 3** The best-fit model (exponential model with exponential-inverse DPI dependency) (curves) compared to observed mortalities against days postinoculation (symbols) from the study of Wang *et al.* (2010) (data set no.3).

and disease burden accurately, it is important to understand the factors determining the transmissibility, infectivity and lethality of HPAI H5N1 viruses. Hemagglutinin (HA) receptor specificity plays an important role in the transmission of influenza viruses, and the affinity of viral HA protein for sialic acid- $\alpha$ 2,6-galactose (SA $\alpha$ 2,6-Gal; human-like receptors) is required for the transmission among ferrets that express SA $\alpha$ 2,6-Gal on respiratory tract

tissues. Recent finding revealed that four influenza virus proteins, not only HA but also PB2, NS1 and PB1-F2, are major determinants of virulence, pathogenicity and host range restriction (Neumann *et al.* 2010). Although only a limited number of dose–response data on HPAI H5N1 virus are available to date, future dose–response analysis on HPAI H5N1 viruses should consider these molecular factors for better understanding of their pathogenesis and



**Figure 4** The best-fit model (exponential model with exponential-inverse DPI dependency) (curves) compared to observed mortalities against days postinoculation (symbols) from the study of van den Brand *et al.* (2010) (data set no.4).

transmissibility among humans. Further QMRA works on HPAI H5N1 viruses will also include the application of the dose-response model by combining with the influenza virus shedding, transportation and exposure models, as described previously (Atkinson and Wein 2008; Nicas and Jones 2009).

In conclusion, we have successfully developed the time-dependent dose-response models of HPAI H5N1 virus, which describe the mortality over time and represent the responses of mice or ferrets accurately. The models developed in the present study, especially the models for ferrets that are excellent model to study human influenza virus infection, will be a useful tool for estimating the time-dependent mortality of HPAI H5N1 virus, for the preparation of a future influenza pandemic caused by this lethal virus.

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