Supplemental Information— Dose Response Parameters for Gain of Function Pathogens

Infection Dose-Response

To quantify the likelihood of an individual or animal becoming infected from exposure to virus, the model uses a probit dose-response function. Experimental infection data from human subjects or animal models of human disease were used to estimate the ID_{50} and probit slope in humans for all virus types—seasonal influenza (including H3N2), pandemic influenza (including H1N1), avian influenza (including H5N1), MERS CoV, and SARS CoV. Additionally, infection parameters were estimated for avian influenza strains in several bird species. Avian infection by human-transmitted viruses was not modeled, because the primary concern in this risk assessment focuses on human consequences.

Human Seasonal Influenza Infection

Carrat et al. (2008) reviewed 56 studies describing the course of influenza in human challenge studies using placebo-treated and untreated volunteers.[1] Included in the review were several studies of seasonal H3N2 infection of human volunteers. The data, however, involved high doses of virus ($\geq$ 1,000 $TCID_{50}$) and resulted in high rates of infection (all doses tested resulted in $\geq$ 50% infection; 18/23 inoculations resulted in $\geq$ 80% infection, including 10 that resulted in 100% infection). A probit model was fit to the data using maximum likelihood estimation (MLE), but due to the heavily skewed data, the resulting curve was very shallow (slope = 0.08) and included an unrealistically low ID_{50} ($< 10^{-10}$ $TCID_{50}$).

The data were further analyzed without the 100% infection results in order to focus on the more informative data points. The probit model fit to these censored data had a slope of 0.217 and an ID_{50} of 3 $TCID_{50}$. In an attempt to rescue the omitted data, each point with a 100% infection rate was modified to assume one infection in twice the number of subjects. The probit model fit to these modified data was shallower (slope = 0.129), because most of the 100% data points occurred at moderate doses; the estimated ID_{50} was again unreasonably low at 6.4×10^{-4}. Both analyses are presented in Figure S1.

[1] Carrat F *et al.* (2008) Time lines of infection and disease in human influenza: a review of volunteer challenge studies. *American journal of epidemiology* 167**:** 775-785

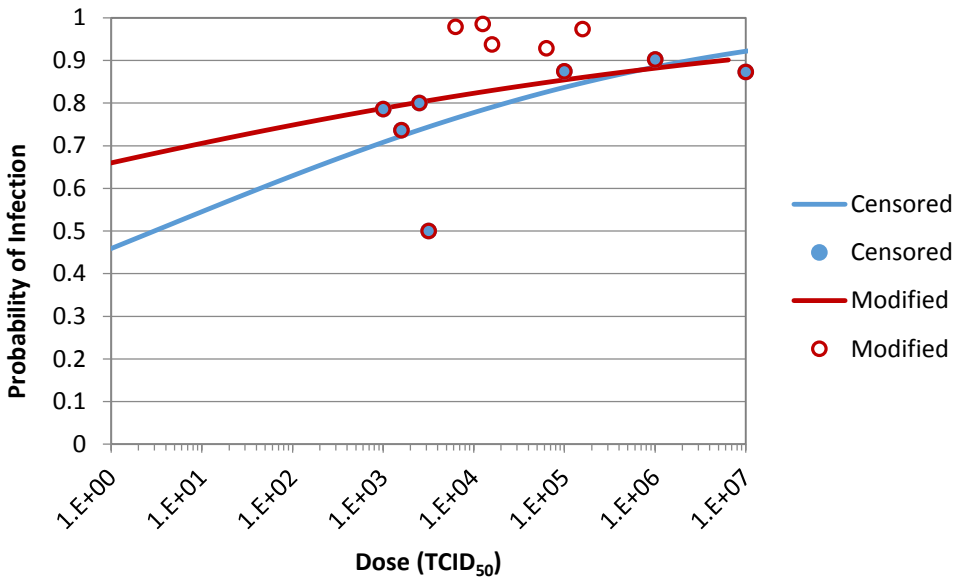


Figure S1. Dose response data and probit fits for H3N2 influenza.

Other studies have reported on seasonal flu infectiousness, which can be used to further inform the probit analysis. Alford et al. (1966) estimated the human inhalational ID_{50} of the H3N2 seasonal virus to be 0.3-6 $TCID_{50}$.[2] Multiple studies have estimated human seasonal flu ID_{50} values using ferret and guinea pig models of infection, all of which reported an aerosol or intranasal ID_{50} of five or fewer PFU.[3,4,5] Based on the above analysis of human infection data, in conjunction with additional reports of low median infectious doses, it appears that the ID_{50} of seasonal influenza is less than 10 PFU. In the model, the range is represented as a uniform distribution from one to five PFU; the probit slope is modeled as a uniform distribution from 0.129 to 0.217 (Table S1).

Table S1. Parameter Description: Human Seasonal Influenza Infectiousness

Parameter	ID_{50}	Probit Slope
Function	Uniform	Uniform
Minimum	1	0.129
Maximum	5	0.217
Mean	3	0.173
Standard Deviation	1.15	0.0254

Human Pandemic Influenza Infection

Of the 56 studies reviewed by Carrat et al. (2008), 29 reported dose response data for influenza A virus subtype H1N1, using intranasal inoculation.[6] These data, along with more recently published data from

[2] Alford RH *et al.* (1966) Human influenza resulting from aerosol inhalation. *Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine* 122**:** 800-804

[3] MacInnes H *et al.* (2011) Transmission of aerosolized seasonal H1N1 influenza A to ferrets. *PLoS One* 6**:** e24448

[4] Gustin KM *et al.* (2011) Influenza virus aerosol exposure and analytical system for ferrets. *Proc Natl Acad Sci U S A* 108**:** 8432-8437

[5] Lowen AC *et al.* (2006) The guinea pig as a transmission model for human influenza viruses. Ibid. 103**:** 9988-9992

[6] Carrat F *et al.* (2008) Time lines of infection and disease in human influenza: a review of volunteer challenge studies. *American journal of epidemiology* 167**:** 775-785

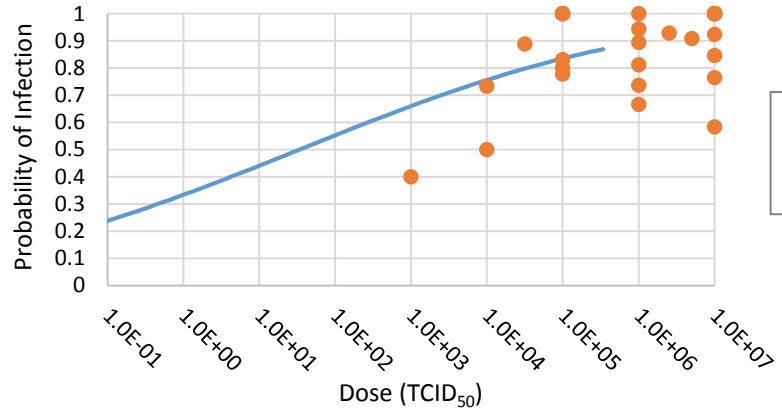
Memoli et al. (2015), were used for a probit analysis.[7] Much like the H3N2 data, however, the H1N1 data involved high doses of virus (all but one inoculum were ≥ 10,000 $TCID_{50}$) and resulted in high rates of infection (all but one tested dose resulted in ≥ 50% infection; 22/30 inoculations resulted in ≥ 80% infection, including 12 that resulted in 100% infection). The probit model fit to the data had a slope of 0.281, and an estimated ID_{50} of 34 $TCID_{50}$. It is unlikely that this is the true ID_{50}, however; the lowest doses tested (10^3 to 10^4 $TCID_{50}$) resulted in 40 to 73% infection, therefore this dose range is more likely to contain the true ID_{50}. The MLE methodology used to fit the probit function was heavily influenced by 96% of the data which were from higher doses that resulted in high infection rates. Therefore, additional analyses were performed in an attempt to identify a more reasonable estimate of the ID_{50}.

To avoid the weighting of the MLE, all data at each unique dose were combined into single data points depicting the percentage of infected subjects at that dose, and a probit model was fit to the data. The resulting function had a slope of 0.370 and an ID_{50} of 408. Memoli et al. specifically reported results using five doses ranging from 10^3 $TCID_{50}$ to 10^7 $TCID_{50}$, with 40% to 85% of volunteers becoming infected. Fitting a probit function to only these data resulted in a slope of 0.371, and an estimated ID_{50} of 2,340 $TCID_{50}$. The similarity of these two analyses is not surprising, as the Memoli data accounted for the majority of the lower-dose subjects. All three analyses are shown in Figure S2.

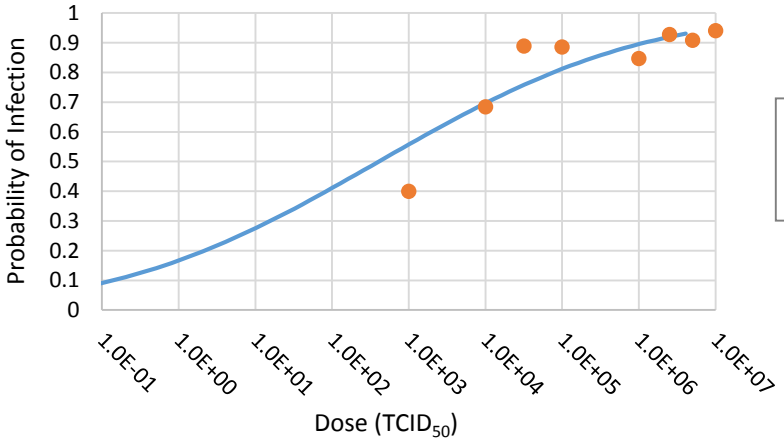
Given the three analyses presented, it is likely that the ID_{50} of H1N1 influenza in humans is between 408 and 2,340 $TCID_{50}$. Assuming a single $TCID_{50}$ is equal to approximately 0.7 PFU,[8] the H1N1 influenza intranasal ID_{50} was represented in the model by a uniform distribution from 286 to 1,638 PFU; The probit slope is modeled as a uniform distribution from 0.281 to 0.371 (Table S2).

[7] Memoli MJ *et al.* (2015) Validation of the wild-type influenza A human challenge model H1N1pdMIST: an A(H1N1)pdm09 dose-finding investigational new drug study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 60**:** 693-702

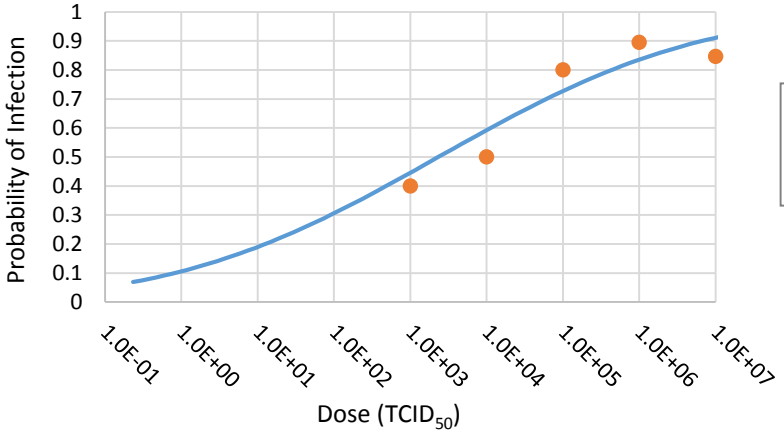
[8] American Type Culture Collection (ATCC). Converting TCID[50] to plaque forming units (PFU) http://www.atcc.org/Global/FAQs/4/8/Converting%20TCID50%20to%20plaque%20forming%20units%20PFU-124.aspx. Last Update 7/25/2012. Accessed 8/3/2015.



All Data (MLE)
Probit slope: 0.281
ID_{50}: 34 $TCID_{50}$



All data (unweighted)
Probit slope: 0.370
ID_{50}: 408 $TCID_{50}$



Memoli et al., 2015
Probit slope: 0.371
ID_{50}: 2340 $TCID_{50}$

Figure S2. Dose response data and three probit fits for H1N1 influenza.

Table S2. Parameter Description: Human Pandemic Influenza Infectiousness		
Parameter	**ID_{50}**	**Probit Slope**
Function	Uniform	Uniform
Minimum	286	0.281
Maximum	1,637	0.371
Mean	962	0.326
Standard Deviation	390	0.0260

Human Infection with Avian Influenza

No data could be found on infection rates of humans (or non-human primates) with avian influenza strains. Given the relative rarity of such infections naturally, it is likely that the median infectious dose is high. In the absence of more specific data, the human infection parameters of avian influenza virus are modeled using the parameters for human pandemic influenza infection.

Avian Influenza Infection

Although no dose-response data could be found for avian influenza infection of birds, one study reported estimated median infectious doses in multiple bird species. Aldous et al. (2010) reported ID_{50} values for both H5N1 and H7N1 avian influenza viruses in various bird species infected both intraocularly and intranasally.[9] In both virus strains, the ID_{50} was much higher in chickens than turkeys. The ID_{50} in chickens was 2.5×10^3 embryo infectious doses (EID_{50}) for H5N1 and 4.0×10^4 EID_{50} for H7N1. For turkeys, the H5N1 and H7N1 ID50s were 10 and 160 EID_{50}, respectively. Ducks demonstrated greater differences in susceptibility to infection by the two virus strains, with an ID_{50} of less than 10 EID_{50} for H5N1 (similar to turkeys), and $\leq 1.6 \times 10^4$ EID_{50} for H7N1 (on the same order of magnitude as chickens).

Because of the great variability in infectious doses among bird species, avian influenza infection of birds is modeled in two ways. Turkeys and other highly susceptible species are modeled with a uniform distribution of ID_{50} from 1 to 10 PFU, while chickens and other less susceptible species are modeled with an ID_{50} of 2500 PFU. Because no dose-response data were available to which a probit model could be fit, the entire probit slope range for combined human seasonal and pandemic influenza (0.129 to 0.217) was used for avian influenza infection (Table S3).

Table S3. Parameter Description: Avian Influenza Infection			
Parameter	**Chicken ID_{50}**	**Other Poultry ID_{50}**	**Probit Slope**
Function	Constant	Uniform	Uniform
Minimum	N/A	1	0.129
Maximum	N/A	10	0.371
Mean	2,500	5.5	0.250
Standard Deviation	N/A	2.6	0.0699

SARS CoV and MERS CoV Infection

Appropriate dose-response studies of infection haven't been conducted for SARS and MERS CoV, representing a considerable gap in the knowledge on these two agents. The lack of literature on the infectious dose of SARS-CoV and MERS-CoV meant looking towards other human coronavirus models to find dose-response data. One study on the human coronavirus 229E, implicated as one of the numerous

[9] Aldous EW *et al.* (2010) Infection dynamics of highly pathogenic avian influenza and virulent avian paramyxovirus type 1 viruses in chickens, turkeys and ducks. *Avian pathology : journal of the WVPA* 39**:** 265-273

agents causing the common cold, measured the dose response to this virus in humans.[10] Adult volunteers were intranasally exposed to varying doses (*n* between 5 and 9 per dose) of 229E virus and were monitored for the development of cold symptoms. A probit model was fit to these data using MLE, resulting in an estimated ID_{50} of 11 $TCID_{50}$ and a probit slope of 1.34 (Figure S3).

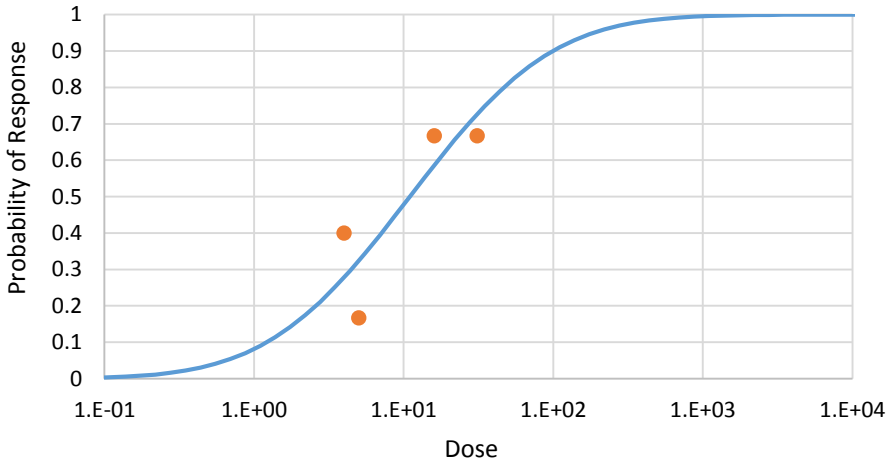


Figure S3. Infectivity probit curve for intranasal 229E virus exposure.

While studies demonstrating the dose-response nature of infection with MERS or SARS CoV were not available, some studies using mouse models have reported on mortality outcomes. While MERS and SARS may not have 100% mortality in all cases, and thus the LD_{50} may be an overestimate of ID_{50}, using mortality data can at least provide an upper bound to the ID_{50} estimate. DeDiego et al. (2008) challenged transgenic mice to four intranasal doses (240; 800; 2,400; and 12,000 PFU) of recombinant SARS-CoV, measuring mortality as the outcome.[11] The three highest doses produced a 100% mortality rate while only one of three mice exposed to 240 PFU died from the infection. In a separate analysis by De Albuquerque et al. (2006), the data from DeDiego were combined with dose-response data from a mouse mortality model of intranasal exposure to mouse hepatitis virus, a related coronavirus.[12] An exponential model was fit to the pooled data, producing an LD_{50} estimate of 280 PFU (95% CI: 130-530 PFU). Another mouse mortality model suggests an LD_{50} < 230 PFU after intranasal doses of 2,300 PFU and 230 PFU resulted in a mortality rate of 100% and 83.3%, respectively.[13] The dose-response data from all three studies is summarized in Table.

For the current analysis, data from all three mouse mortality studies were pooled and a probit model was fit using MLE, resulting in an estimated LD_{50} of 162 PFU and a probit slope of 1.79 (Figure S4).

Table S4. Coronavirus Dose-Response Mortality Data

Study	Dose (PFU)	Mortality	Details
	240	1/3 (33.3%)	Intranasal, transgenic mice
	800	3/3 (100.0%)	
	2,400	2/2 (100.0%)	

[10] Bradburne AF *et al.* (1967) Effects of a "new" human respiratory virus in volunteers. *British medical journal* 3**:** 767-769

[11] Dediego ML *et al.* (2008) Pathogenicity of severe acute respiratory coronavirus deletion mutants in hACE-2 transgenic mice. *Virology* 376**:** 379-389

[12] Watanabe T *et al.* (2010) Development of a dose-response model for SARS coronavirus. *Risk analysis : an official publication of the Society for Risk Analysis* 30**:** 1129-1138

[13] McCray PB, Jr. *et al.* (2007) Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus. *Journal of virology* 81**:** 813-821

Study	Dose (PFU)	Mortality	Details
DeDiego ML et al. (2008)[14]	12,000	6/6 (100.0%)	
De Albuquerque N et al. (2006)[15]	5	0/5 (0.0%)	Intranasal mouse hepatitis virus, mice
	50	1/5 (20.0%)	
	500	3/5 (60.0%)	
	5,000	5/5 (100.0%)	
McCray PB et al. (2007)[16]	230	5/6 (83.3%)	Intranasal, mice
	2,300	3/3 (100.0%)	

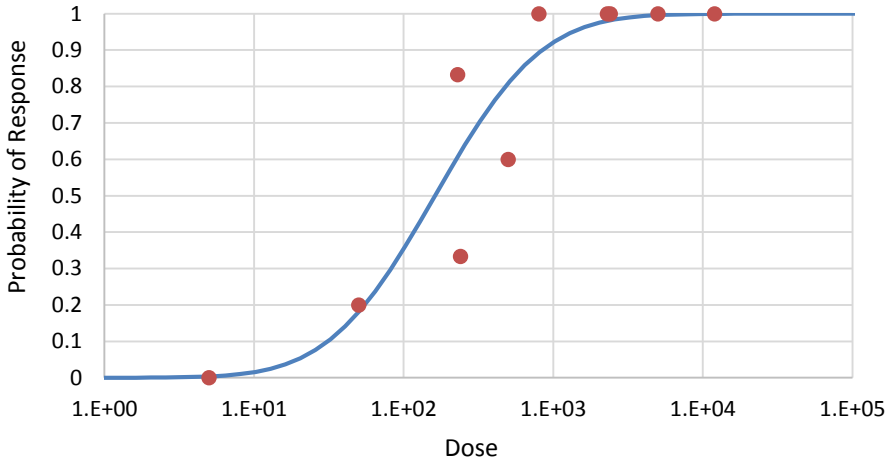


Figure S4. Mortality probit curve for intranasal coronavirus exposure.

Few studies utilizing animal models exist for MERS, none of which investigate dose response to the virus to allow for calculating its ID_{50}. As MERS-CoV is a coronavirus related to and capable of producing an illness with a severity similar to SARS-CoV, the ID_{50} of MERS-CoV is presumed to be similar to that of SARS-CoV. Therefore, both viruses are modeled with the same infection parameters. The ID_{50} of the coronaviruses is modeled using a log-triangle distribution, with a minimum of 11 PFU, mode of 162 PFU, and maximum of 530 PFU (from the estimated 229E virus ID_{50}, the estimated SARS CoV LD_{50}, and the upper confidence interval of the SARS CoV LD_{50} as reported by De Albuquerque et al.); a probit slope of 1.34 is used for all simulations (Table).

Table S5. Parameter Description: SARS CoV and MERS CoV Infection		
Parameter	**ID_{50}**	**Probit Slope**
Function	Log-triangle	Constant
Minimum	11	N/A
Maximum	530	N/A
Mean	99	1.34
Standard Deviation	N/A	N/A

[14] Dediego ML *et al.* (2008) Pathogenicity of severe acute respiratory coronavirus deletion mutants in hACE-2 transgenic mice. *Virology* 376**:** 379-389

[15] De Albuquerque N *et al.* (2006) Murine hepatitis virus strain 1 produces a clinically relevant model of severe acute respiratory syndrome in A/J mice. *Journal of virology* 80**:** 10382-10394

[16] McCray PB, Jr. *et al.* (2007) Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus. Ibid. 81**:** 813-821