Medical Countermeasure Models Volume 8: Botulinum Neurotoxin

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14. ABSTRACT
The botulinum neurotoxin medical countermeasure model presented here allows users to explore how medical countermeasures (MCM) can impact the course of the disease, mortality, and loss of work. The model is designed to allow users to input information about exposure and countermeasures, run a simulation and display outputs. The parameters describing disease outcomes for untreated patients that underlie the model are taken from AMedP-8(C), while the parameters describing the efficacy of MCM were established specifically for this project using publicly available data from human and animal studies. This stochastic model allows users to input data about each exposed individual including the inhaled dose, pre-exposure vaccine status, and treatment status and timing. After the model is run, the output tab displays the outcome for each individual. The graph tab provides a summary of the results, including the percent of individuals that die, recover, or never develop illness, as well as the time distributions of symptom onset and death. The sample results included in this report demonstrate how MCM can impact the number of casualties, the timing of the disease, and the number of days of work lost. Users of the model can explore additional scenarios by modifying the dose and MCM inputs.

15. SUBJECT TERMS
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Preface

The research and development work described in this report was conducted by Gryphon Scientific, LLC for the Joint Science and Technology Office (JSTO) of the Department of Defense (DoD) Chemical and Biological Defense (CBD) Program. JSTO is also the Chemical/Biological Technologies (CB) Directorate in the Research and Development (RD) Enterprise of the Defense Threat Reduction Agency (DTRA). Contract HDTRA1-10-C-0025 is titled *Medical Countermeasures for CBR Agents*.

This project was initiated by Ms. Nancy Nurthen of the Information Systems Capability Development Division (RD-CBI), and was transitioned to Dr. Christopher Kiley at RD-CBI for the first option year. It was funded under DTRA Contract Number HDTRA1-10-C-0025 to Gryphon Scientific, LLC, with subcontractor Applied Research Associates, Inc. (ARA). The target application for the product of this contract is To Be Determined (TBD) under the auspices of the Joint Project Manager for Information Systems (JPM IS) of the Joint Program Executive Office for Chemical and Biological Defense (JPEO-CBD).

Table of Contents

Executive Summary

The botulinum neurotoxin medical countermeasure model presented here allows users to explore how medical countermeasures (MCM) can impact the course of the disease, mortality, and loss of work. The model is designed to allow users to input information about exposure and countermeasures, run a simulation and display outputs. The parameters describing disease outcomes for untreated patients that underlie the model are taken from AMedP-8(C), while the parameters describing the efficacy of MCM were established specifically for this project using publicly available data from human and animal studies. This stochastic model allows users to input data about each exposed individual including the inhaled dose, pre-exposure vaccine status, and treatment status and timing. After the model is run, the output tab displays the outcome for each individual. The graph tab provides a summary of the results, including the percent of individuals that die, recover, or never develop illness, as well as the time distributions of symptom onset and death. The sample results included in this report demonstrate how MCM can impact the number of casualties, the timing of the disease, and the number of days of work lost. Users of the model can explore additional scenarios by modifying the dose and MCM inputs.

Introduction

Accurate modeling of medical countermeasure efficacy against chemical, biological and radiological (CBR) agents is essential to understanding the vulnerabilities of our warfighters on the modern battlefield. In helping calculate the benefit of countermeasures, modeling can inform data-driven purchasing decisions and logistical tradeoffs. In this study, Gryphon Scientific and Applied Research Associates (ARA) developed models to predict the efficacy of medical countermeasures against a variety of agents.

This report (prepared by Gryphon Scientific) is one of ten describing the medical countermeasure models constructed for this project. This volume focuses exclusively on the methods used to construct the botulinum neurotoxin model, instructions on how to use the model, and examples of the outputs generated by the model. Other volumes describe models for *B. anthracis* (volume 1), organophosphates (volume 2), cesium-137 (volume 3), *F. tularensis* (volume 4), sulfur mustard (volume 5), americium-241 (volume 6), *Y. pestis* (volume 7), plutonium-238/239 (volume 9), and vesicants (volume 10, an expansion on volume 5). Each volume begins by briefly introducing the modeled agent and the countermeasures available against the agent. The overall schematic of each model and the relevant parameters are then discussed, along with a brief explanation of the rationale for selecting each parameter. Lastly, this report discusses the calculations and computational framework of the Microsoft Excel model and provides examples of modeling outputs.

Summary of Deliverables

Below is a description of the four deliverables assigned for each agent of interest, and a description of what is included in each deliverable. The item in bold (number four, the "MCM Model Built in Microsoft Excel") is the deliverable presented in this final report.

1. Modeling Approach

   The modeling approach deliverable describes each of the parameters that we anticipate including in our MCM model. For each parameter, a description of the approach for developing and justifying the parameter is presented. The approach developed is based on prior knowledge of the agent and on the general types of data available for each agent, but specific citations are not included as it is a preliminary document.

2. Modeling Parameters

   The modeling parameters deliverable defines the value or function for each parameter used to develop the MCM model. Each parameter is supported with a description of the rationale for choosing the parameter, including any scientific evidence used in parameter development or assumptions that were made.

3. MCM Model

   In addition to the information already developed in the "Modeling Parameters" deliverable, the "MCM Model" includes a description of the model, user inputs, the model calculations, and the model outputs. For the biological agents, the report is an accompaniment to a preliminary implementation of the MCM model built in Microsoft Excel.

4. MCM Model Built in Microsoft Excel

Microsoft Excel is used for the final implementation of the biological MCM models. This implementation of the model includes feedback and adjustments made after review of the previous deliverables, and will be available for independent verification and validation.

Botulinum Neurotoxin

Overview

Botulinum neurotoxin is produced primarily by the bacterial species *Clostridium botulinum,* although *Clostridium baratii* and *Clostridium butyricum* are also capable of producing neurotoxins that cause botulism.[1] There are seven different antigenic types of botulinum neurotoxin, named A through G; exposure to Type A leads to the most severe form of the disease.[2] Botulinum neurotoxin inhibits the release of neurotransmitters and given a sufficient dose, can cause death through a descending, flaccid paralysis which can reach the respiratory muscles. When death does not occur, recovery from paralysis is possible, but can take weeks to months since new connections between nerves and muscles must be established.[3,4]

Most botulism cases in the clinical literature are categorized as food-borne botulism, wound botulism, or intestinal botulism (adult and infant). Food-borne botulism results from the ingestion of toxin present in food items,[5] while wound botulism and intestinal botulism result from an infection with toxin-producing *Clostridium* bacteria.[6] However, once botulinum neurotoxin is absorbed into the bloodstream, the symptoms of all types of botulism are virtually the same.[7]

In a battlefield situation, soldiers are most likely to be exposed to the agent via a fourth route, inhalation of the aerosolized toxin; however, data on inhalational botulism in humans are very scarce. Inhalation of botulinum neurotoxin results in exposure to a single dose of toxin rather than gradual production of the toxin at an infection site, as occurs with wound and intestinal botulism. In human cases of food-borne botulism and animal studies with injected toxin, disease can also occur from a single dose of toxin; therefore, experts believe that the disease course following inhalational exposure would be similar to that resulting from other single-dose exposures.[8] Thus, the parameters described in this report were developed primarily using data from human food-borne cases of botulism and animal studies using injected toxin.

Countermeasures

The countermeasures against botulinum neurotoxin include pre-exposure vaccination, post-exposure treatment with antitoxin, mechanical ventilation and other supportive care. Until recently, the investigational Pentavalent (ABCDE) Botulinum Toxoid (PBT) vaccine was available to botulism researchers and at-risk military personnel. The vaccine was discontinued in November of 2011 due to declining immunogenicity and increased occurrence of moderate adverse reactions in recent years.[9,10] The primary treatment for non-vaccinated individuals and individuals for whom vaccination does not prevent

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[1] Arnon SS et al. "Botulinum toxin as a biological weapon." *Journal of the American Medical Association.* **285**(1059). 2001.
[2] Arnon SS et al. "Botulinum toxin as a biological weapon." *Journal of the American Medical Association.* **285**(1059). 2001.
[3] Arnon SS et al. "Botulinum toxin as a biological weapon." *Journal of the American Medical Association.* **285**(1059). 2001.
[4] McAdam AJ and Sharpe AH. "Infectious Diseases." *Pathologic Basis of Disease.* 7th Edition. Elsevier Saunders, Philadelphia. 2005.
[5] CDC "Facts about Botulism." http://www.bt.cdc.gov/agent/botulism/factsheet.asp. Accessed on Nov 17, 2011.
[6] Arnon SS et al. "Botulinum toxin as a biological weapon." *Journal of the American Medical Association.* **285**(1059). 2001.
[7] Arnon SS et al. "Botulinum toxin as a biological weapon." *Journal of the American Medical Association.* **285**(1059). 2001.
[8] Donaghy M. "Neurologists and the threat of bioterrorism." *Journal of the Neurological Sciences.* **249**(1). 2006.
[9] Smith LA. "Botulism and vaccines for its prevention." *Vaccine.* **27**(Suppl 4). 2009.
[10] CDC. "Notice of CDC's Discontinuation of Investigational Pentavalent (ABCDE) Botulinum Toxoid Vaccine for Workers at Risk for Occupational Exposure to Botulinum Toxins." *Morbidity and Mortality Weekly Report.* October 28, 2011. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6042a3.htm. Accessed on December 15, 2011.

the onset of symptoms includes the investigational heptavalent botulinum antitoxin (HBAT).[11,12] HBAT cannot reverse existing paralysis, although it can reduce further damage from the toxin.[13,14,15] In addition to antitoxin, treatment of botulism typically requires supportive care, which we define as the use of medical supplies commonly available in a hospital, such as feeding tubes and intravenous fluids.[16] Because botulism deaths typically occur as a result of respiratory failure, mechanical ventilation is often used in conjunction with antitoxin treatment; therefore mechanical ventilation is also included in our treatment parameters.[17]

Botulinum antitoxin is currently not administered as post-exposure prophylaxis (PEP) before the onset of symptoms due to the risk of adverse effects and the high cost of the countermeasure. Instead, asymptomatic individuals that are suspected to have been exposed to botulinum neurotoxin are closely monitored for signs of symptom onset.[18]

Model Overview

We developed a stochastic model of the efficacy of medical countermeasures (MCM) against botulinum neurotoxin. Given a description of agent exposure, vaccination, and treatment, the model calculates the outcome for each individual in terms of morbidity, mortality, loss of work due to illness, and any adverse effects due to medical countermeasures. The evidence-based parameters, which form the basis of the model, determine the probability of each outcome, and the model draws a random number to determine which outcome is realized for any individual by comparing the random number to the probability of the outcome.

A schematic of the botulism MCM model illustrates where each piece of data is applied and how the model functions (Figure 1). Inputs are indicated by light blue ovals and include dose of agent, vaccination inputs, and treatment inputs. Each input feeds directly into the modeling calculations indicated by dark blue rectangles. Purple rectangles represent intermediate outcomes, while terminal model outputs are represented by red rectangles and include death, survival with no loss of work, or survival with loss of work.

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[11] Fagan RP et al. "Initial recovery and rebound of Type F intestinal colonization botulism after administration of investigational heptavalent botulinum antitoxin." *Clinical Infectious Diseases.* E-publication ahead of print. September 6, 2011.

[12] The FDA has approved a human-derived botulism immune globulin (derived from human serum), but the use of this product is limited to treatment of infant botulism in patients less than one year of age; therefore this antitoxin is not included in our analysis. Citations for these statements follow: *Thanongsaksrikul, J and Chaicumpa W. "Botulinum Neurotoxins and Botulism: A Novel Therapeutic Approach." *Toxins.* 3. 2011.*Arnon SS et al. "Botulinum toxin as a biological weapon: medical and public health management." *Journal of the American Medical Association.* **285**(8). 2001. *MassBioLogics, Cangene Corporation, FFF Enterprises. BabyBIG [Botulism Immune Globulin Intravenous (Human) (BIG-IV)]. *Package Insert.* September 2009.

[13] Sobel J. "Botulism." *Clinical Infectious Diseases.* **41**(1167-1173). 2005.

[14] Chalk C, Benstead TJ, and Keezer M. "Medical treatment for botulism (Review)." *Cochrane Database of Systematic Reviews: CD008123.* 2011.

[15] Dembek Z, Smith L, and Rusnak J. "Botulinum toxin."*Medical Aspects of Biological Warfare.* 337-353. 2007.

[16] Arnon SS et al. "Botulinum toxin as a biological weapon." *Journal of the American Medical Association.* **285**(1059). 2001.

[17] Arnon SS et al. "Botulinum toxin as a biological weapon." *Journal of the American Medical Association.* **285**(1059). 2001.

[18] Shapiro RL et al. "Botulism in the United States: a clinical and epidemiologic review." *Annals of Internal Medicine.* **129**(221-228). 1998.
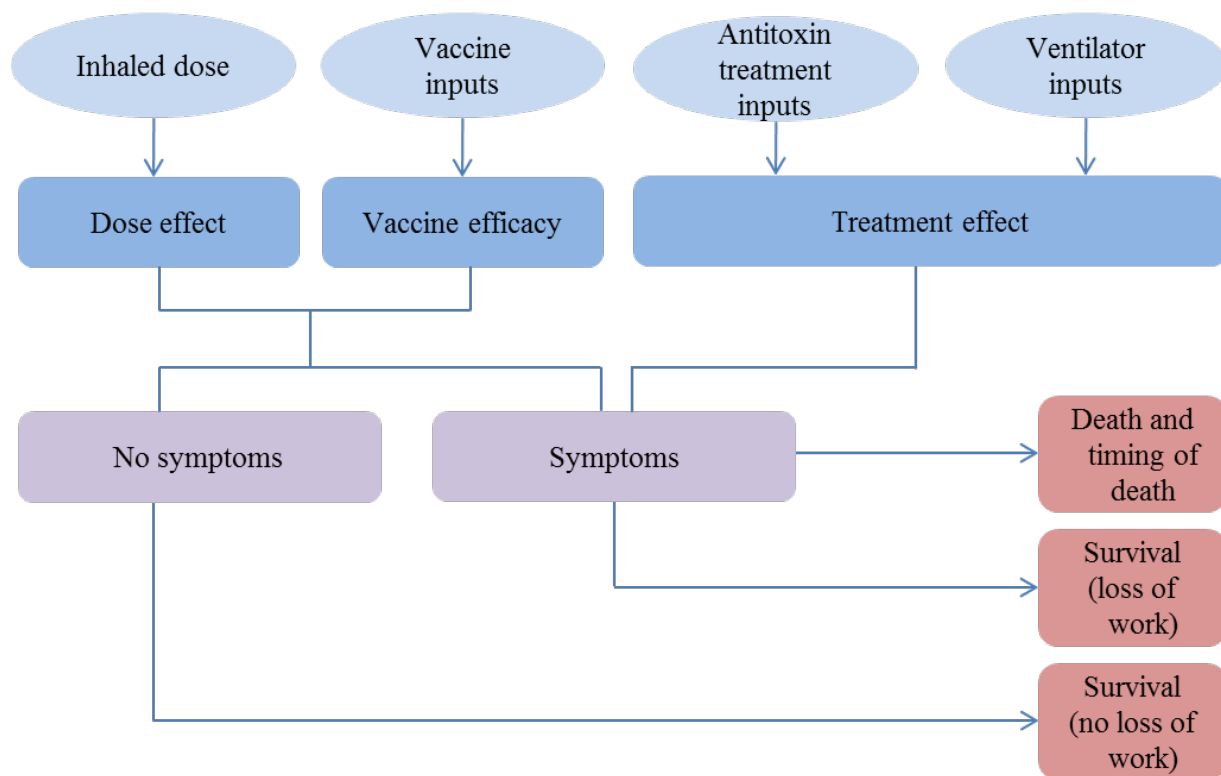
Figure 1. Botulism modeling scheme. Blue circles indicate user inputs, blue rectangles indicate parameters, purple rectangles are intermediate steps and red rectangles are terminal model outputs.

Microsoft Excel Model Overview

The model operates in Microsoft Excel and has four tabs available to users: "Inputs," "Outputs," "Graphs," and "Advanced User." The modeling calculations are located on tabs hidden from the user. On the "Inputs" tab, exposure and MCM conditions can be entered separately for each individual (Figure 2). To understand outcomes for a group of individuals, the user should use the "copy and paste" tool to input multiple individuals with the same characteristics. For example, given data from 10,000 cases in which 50% were treated with antibiotics, 5,000 would be entered as identical treated individuals and 5,000 would be entered as identical untreated individuals.

	A	B	C	D	E	F	G	H	I	J
1	Patient #	Inhaled dose (ng)	Pre exposure vaccine	Incomplete vaccination: weeks prior to exposure that the last dose was administered	Treatment	Treatment time: days after onset of symptoms				
2	1	10,000	NA	NA	Antitoxin + ventilation	1				
3	2	1,000	NA	NA	NA	NA				
4	3	12	Complete	NA	NA	NA				
5	4	15	NA	NA	Antitoxin alone	3				
6	5	260	Complete	NA	Antitoxin + ventilation	5				
7	6	600	Incomplete	1	NA	NA				
8	7	8,000	Incomplete	5	NA	NA				
9	8	12,000	NA	NA	Antitoxin alone	1				
10	9	120	Complete	NA	Antitoxin alone	0				
11	10	100,000	NA	NA	NA	NA				
12	11	150,000	Complete	NA	Antitoxin alone	10				
13	12	150	Incomplete	7	Antitoxin + ventilation	3				
14	13	8,000	Incomplete	12	NA	NA				
15	14	800,000	NA	NA	NA	NA				
16	15	16	NA	NA	NA	NA				
17	16	10	Incomplete	5	Antitoxin alone	7				
18	17	1,000	Complete	NA	Antitoxin + ventilation	5				
19	18	12	NA	NA	NA	NA				
20	19	15	Complete	NA	NA	NA				
21	20	260	Incomplete	5	Antitoxin alone	4				
22	21	80,001	Incomplete	6	Antitoxin alone	6				
23	22	120	NA	NA	NA	NA				
24	23	100,000	Complete	NA	Antitoxin alone	3				
25	24	150,000	NA	NA	Antitoxin + ventilation	2				
26	25	150	NA	NA	NA	NA				
27	26	8,000	Complete	NA	NA	NA				
28	27	800,000	NA	NA	NA	NA				
29	28	16	NA	NA	Antitoxin alone	12				
30	29	8,000	Complete	NA	Antitoxin alone	14				
31	30	12,000	NA	NA	Antitoxin + ventilation	1				
32	31	120	NA	NA	NA	NA				
33	32	120,000	Complete	NA	NA	NA				
34	33	8,000	Incomplete	14	Antitoxin alone	6				

Run Model

Input / Outputs / Graphs / Advanced User

Figure 2. Screen shot of "Inputs" tab.

Table 1, below, gives detailed information about each of the user input options shown in Figure 2 and explains how each input should be used.

Table 1. MCM Model Inputs	
Input Category	**Explanation of Input**
Dose of Agent	
Inhaled dose	Designates the dose (in ng) of inhaled botulinum neurotoxin.
Vaccination Inputs	
Pre-exposure vaccine	Designates whether or not an individual receives a vaccine prior to exposure. The "Complete" vaccination option indicates that the entire recommended vaccine regimen, which consists of four doses at weeks 0, 2, 12 and 26, plus annual boosters, where the last booster (or dose) is received within four years of exposure.[19] The "Incomplete" vaccination option indicates that only the first three doses were administered. If vaccine is not administered, the user must select "NA" from the dropdown menu.
Incomplete vaccination: weeks prior to exposure that the last dose was administered	Designates the time, in weeks before exposure, that the third vaccine dose is administered when "Incomplete" vaccination is selected as the "Pre-Exposure Vaccine" input. If "NA" or "Complete" is selected for the "Pre-Exposure Vaccine" input, "NA" may be entered in the "weeks prior to exposure" input; any numbers entered for this input will be ignored. If "Incomplete" is selected, then a number of weeks must be entered; entering "NA" will result in an error. If zero is entered or this field is left blank, the model will give the same result as an unvaccinated individual.
Treatment Inputs	
Treatment	Designates whether or not treatment is available for the individual should symptoms develop. If a treatment is selected but the model determines the individual will not develop symptoms, the treatment is not used. The "Antitoxin + ventilation" option indicates that antitoxin and supportive care are available, and mechanical ventilation assistance will be provided if respiratory paralysis occurs. The "Antitoxin alone" option indicates that antitoxin and supportive care are available, but mechanical ventilation is not available. If treatment is not available, the user must select "NA" from the dropdown menu.
Treatment time: days after onset of symptoms	If a treatment is available (either "Antitoxin + ventilation" or "Antitoxin alone"), this input designates how many days after the onset of symptoms the individual first receives treatment. The user may select integer values for treatment time from a dropdown menu. If the treatment time is left blank, the model will assume that treatment is started on day zero, the day that symptoms first appear. If no treatment is selected the user may select "NA" for this field; any values input when no treatment is selected will be ignored.

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[19] We did not develop a modeling parameter for vaccine efficacy when individuals received the full vaccine course more than four years before exposure. Although these individuals are likely to have some protection, we found no data that could be used to support parameter development. Therefore, the modeling input page does not allow users to choose complete vaccination more than four years prior to exposure. Users who select complete vaccination (which assumes that the last dose or booster was within four years of exposure) as a proxy will likely underestimate the number of causalities, while users who select no vaccination as a proxy will likely overestimate casualties.

Once the user-defined parameters are entered, the "Run Model" button will start the calculations. When the model has finished running, the "Graphs" tab is automatically selected (Figure 3). In this tab, the results are described in a summary box that includes: the total number of individuals exposed to botulinum neurotoxin, the number that develop symptoms, the number that die, the number of individuals who develop respiratory paralysis (survivors and non-survivors), the number of survivors who return to work, the days of work lost for survivors who return to work, the number of survivors who will be unable to return to work within six months, and the number of survivors with adverse effects from the treatment. This tab also includes a pie chart of the outcomes (percent dead, recovered, and not sick) and scatter plots displaying the daily totals of how many people develop symptoms and how many people die each day.

Users who wish to view a more detailed account of each individual's outcome can select the "Outputs" tab (Figure 4). Here, the user can again view the summary box seen on the "Graphs" tab. Results for each individual are also presented, including whether or not symptoms occurred, time of symptom onset (if applicable), duration of symptoms (if applicable), whether or not respiratory paralysis occurred, outcome (recovered, dead or no symptoms), time to death (if applicable), days of work lost due to illness (if applicable), and whether or not treatment adverse effects occurred (hives, serum sickness, and anaphylaxis).
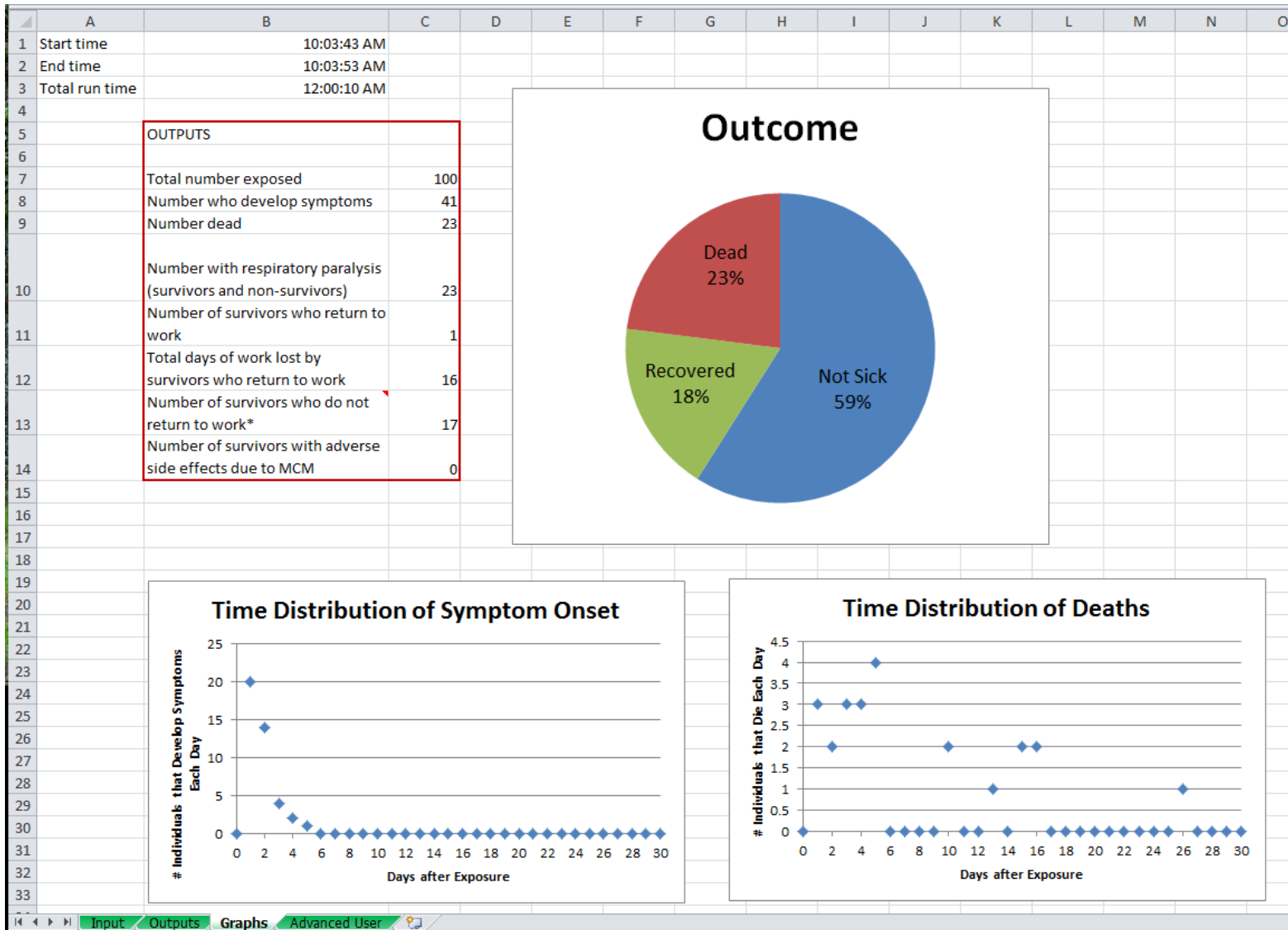
Figure 3. Screen shot of "Graphs" tab.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1					Patient #	Symptoms	Time of Symptom Onset	Duration of Symptoms	Respiratory Paralysis	Outcome	Time to Death	Days of Work Lost	Hives	Serum Sickness	Anaphylaxis
2		OUTPUTS			1	Symptoms	1	3	Yes	Dead	4	NA	NA	NA	NA
3					2	Symptoms	3	1	Yes	Dead	4	NA	NA	NA	NA
4		Total number exposed	100		3	No Symptoms	NA	NA	NA	No Symptoms	NA	NA	NA	NA	NA
5		Number who develop symptoms	41		4	No Symptoms	NA	NA	NA	No Symptoms	NA	NA	NA	NA	NA
6		Number dead	23		5	No Symptoms	NA	NA	NA	No Symptoms	NA	NA	NA	NA	NA
7		Number with respiratory paralysis (survivors and non-survivors)	23		6	Symptoms	2	>180	No	Recovered	NA	No Return to Work	NA	NA	NA
8		Number of survivors who return to work	1		7	No Symptoms	NA	NA	NA	No Symptoms	NA	NA	NA	NA	NA
9		Total days of work lost by survivors who return to work	16		8	Symptoms	2	13	Yes	Dead	15	NA	NA	NA	NA
10		Number of survivors who do not return to work*	17		9	No Symptoms	NA	NA	NA	No Symptoms	NA	NA	NA	NA	NA
11		Number of survivors with adverse side effects due to MCM	0		10	Symptoms	2	1	Yes	Dead	3	NA	NA	NA	NA
12					11	Symptoms	2	16	No	Recovered	NA	16	No	No	No
13					12	No Symptoms	NA	NA	NA	No Symptoms	NA	NA	NA	NA	NA
14					13	No Symptoms	NA	NA	NA	No Symptoms	NA	NA	NA	NA	NA
15					14	No Symptoms	NA	NA	NA	No Symptoms	NA	NA	NA	NA	NA
16					15	No Symptoms	NA	NA	NA	No Symptoms	NA	NA	NA	NA	NA
17					16	No Symptoms	NA	NA	NA	No Symptoms	NA	NA	NA	NA	NA
18					17	No Symptoms	NA	NA	NA	No Symptoms	NA	NA	NA	NA	NA
19					18	No Symptoms	NA	NA	NA	No Symptoms	NA	NA	NA	NA	NA
20					19	No Symptoms	NA	NA	NA	No Symptoms	NA	NA	NA	NA	NA
21					20	Symptoms	1	>180	No	Recovered	NA	No Return to Work	No	No	No
22					21	No Symptoms	NA	NA	NA	No Symptoms	NA	NA	NA	NA	NA
23					22	Symptoms	1	0	Yes	Dead	1	NA	NA	NA	NA
24					23	No Symptoms	NA	NA	NA	No Symptoms	NA	NA	NA	NA	NA
25					24	Symptoms	2	14	Yes	Dead	16	NA	NA	NA	NA
26					25	No Symptoms	NA	NA	NA	No Symptoms	NA	NA	NA	NA	NA
27					26	Symptoms	2	11	Yes	Dead	13	NA	NA	NA	NA
28					27	Symptoms	4	1	Yes	Dead	5	NA	NA	NA	NA

Input **Outputs** Graphs Advanced User

Figure 4. Screen shot of "Outputs" tab.

The final tab available to users is the "Advanced User" tab. This tab allows users to change many of the modeling parameters including: untreated disease course parameters, vaccine efficacy, treatment efficacy, treated disease course parameters, and many others. Each parameter on this tab has a user-defined value and a recommended value (Figure 5). Users who wish to use the default values defined in this document should ensure that the values in the green "user value" column match those in the red "recommended value" column.

	A	B	C	D	E
4		**Effectivity: Probability of Becoming Ill**	**User Input**	**Recommended Parameter**	**Units/Description**
5		Probit	12.9	12.9	probits/logdose
6		ED50	0.1	0.1	ug/man
7		**Lethality: Probability of Death**			
8		Probit	12.9	12.9	probits/logdose
9		LD50	0.8	0.8	ug/man
10		**Latent Period**			
11		μ	0	0	days
12		σ	0.84	0.84	days
13		**Survivor Illness Durations**			
14		Stage 1 mean	1	1	days
15		Stage 1 standard deviation	1.12	1.12	days
16		Stage 2 mean	14	14	days
17		Stage 2 standard deviation	8.54	8.54	days
18		Stage 3 (constant)	180	180	days
19		**Non-Survivor Illness Duration**			
20		λ	0.318	0.318	
21		**Vaccine Efficacy**			
22		Complete	98%	98%	
23		**Antitoxin + Ventilation Efficacy**			
24		When administered on the same day as symptom onset	78%	78%	
25		When administered one day after symptom onset	72.5%	72.5%	
26		When administered more than than one day after symptom onset	67%	67%	
27		When administered on the day of death	33.5%	33.5%	
28		**Rate of Respiratory Paralysis**			
29		Treated non-survivors	100%	100%	
30		Treated survivors	50%	50%	
31		**Antitoxin Effect on Disease Course in Non-Survivors**			
32		Increase in symptomatic period	400%	400%	
33		Standard deviation of increase	265%	265%	
34		**Rate of Antitoxin Side Effects**			
35		Frequency of hives	2%	2%	
36		Frequency of serum sickness	2%	2%	
37		Frequency of anaphylaxis	1%	1%	

Input / Outputs / Graphs / **Advanced User**

Figure 5. Advanced User tab.

Modeling Assumptions and Parameters

Assumptions: Base Case

Exposure

Botulinum neurotoxin can cause disease via a variety of routes. Aerosolization of the toxin (not the bacteria) is the most likely battlefield scenario. Since botulinum neurotoxin is not absorbed through the skin, exposure via inhalation is the only route of exposure considered in this model.[20]

Serotype

Although there are multiple serotypes of botulinum neurotoxin, AMedP-8(C) assumes exposure to serotype A, which typically causes the most severe form of the disease.[21] Our MCM model is designed to be used in conjunction with AMedP-8(C); therefore our model also assumes exposure to serotype A.

Toxin Dose Effect

Many sources indicate that for botulism, the duration and severity of the disease are dependent on the dose of toxin.[22,23,24] While AMedP-8(C) includes toxin dose-dependence for the "Effectivity" and "Lethality" parameters, it does not incorporate toxin dose-dependence into the "Duration of the Latent Period" and "Duration of the Symptomatic Period" parameters. Since AMedP-8(C) does not incorporate toxin dose into many of its parameters and the available data used to establish our MCM parameters often did not include information about toxin dose, our countermeasure parameters do not directly incorporate the effect of toxin dose.

Vaccine

Although the PBT vaccine was recently discontinued,[25] the vaccination parameters in our model are based on this vaccine. There are a number of other vaccines in development that have reached clinical trials in humans, but because PBT was so recently discontinued we assume that most immunized individuals received the PBT vaccine.[26] The vaccine parameters included in this document are based on PBT, but if advanced users wish to incorporate efficacy data for a newly developed drug, the vaccine parameters are available for modification under the "Advanced User" tab.

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[20] Arnon SS et al. "Botulinum toxin as a biological weapon." *Journal of the American Medical Association*. **285**(1059). 2001.
[21] Woodruff BA et al. "Clinical and Laboratory Comparison of Botulism from Toxin Types A, B, and E in the United States, 1975-1988." *The Journal of Infectious Diseases*. **166**(6). 1992.
[22] MacDonald KL et al. "Type A Botulism from Sauteed Onions. Clinical and Epidemiological Observations." *Journal of the American Medical Association*. **253**(9). 1985.
[23] Arnon SS et al. "Botulinum toxin as a biological weapon." *Journal of the American Medical Association*. **285**(1059). 2001.
[24] Tacket CO et al. "Equine Antitoxin Use and Other Factors that Predict Outcome in Type A Foodborne Botulism." *The American Journal of Medicine*. **76**. 1984.
[25] CDC. "Notice of CDC's Discontinuation of Investigational Pentavalent (ABCDE) Botulinum Toxoid Vaccine for Workers at Risk for Occupational Exposure to Botulinum Toxins." *Morbidity and Mortality Weekly Report.* October 28, 2011. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6042a3.htm. Accessed on December 15, 2011.
[26] Zygmunt F et al. "Botulinum Toxin." *Medical Aspects of Biological Warfare.* Department of Defense, Office of the Surgeon General, US Army, Borden Institute, Washington DC. 2007.

Treatment

This model assumes that all individuals who receive treatment will be administered the investigational heptavalent botulinum antitoxin (HBAT)[27] and will be provided with the supportive care they require (for example, feeding tubes and intravenous fluids). Users of the model may choose to add mechanical ventilation, which could be a limiting resource in a battlefield scenario, to the treatment regimen. However, the use of mechanical ventilation in the absence of antitoxin treatment is not considered in the model because there are not sufficient data available in the literature to establish the efficacy of ventilation support alone (as described in the "Botulism Treatment: Antitoxin and Mechanical Ventilation" section).

Parameters

Untreated Disease Course

The parameters describing the untreated disease course are primarily taken from AMedP-8(C) NATO Planning Guide for the Estimation of CBRN Casualties Ratification Draft 1.[28] This medical countermeasure model is designed to merge with this previously established model of untreated illness and thus will utilize the same parameters. Below, we summarize the untreated modeling parameters established in AMedP-8(C): effectivity, duration of the latent period, lethality, duration of the symptomatic period, and time of death.[29]

Effectivity

The "Effectivity" parameter determines the likelihood of developing symptoms after exposure. As established by AMedP-8(C),[30] the "Effectivity" parameter operates as a function of dose, where the likelihood of symptoms increases with inhaled dose. The schematic shown below (Figure 6) illustrates the data that influence this modeling parameter.

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27 Fagan RP et al. "Initial recovery and rebound of Type F intestinal colonization botulism after administration of investigational heptavalent botulinum antitoxin." *Clinical Infectious Diseases.* E-publication ahead of print. September 6, 2011.
28 NATO. "AMedP-8(C) NATO Planning Guide for the Estimation of CBRN Casualties Ratification Draft 1." February 2010.
29 In a few circumstances the parameters published in AMedP-8(C) were adjusted. All adjustments are described in this document.
30 NATO. "AMedP-8(C) NATO Planning Guide for the Estimation of CBRN Casualties Ratification Draft 1." February 2010.
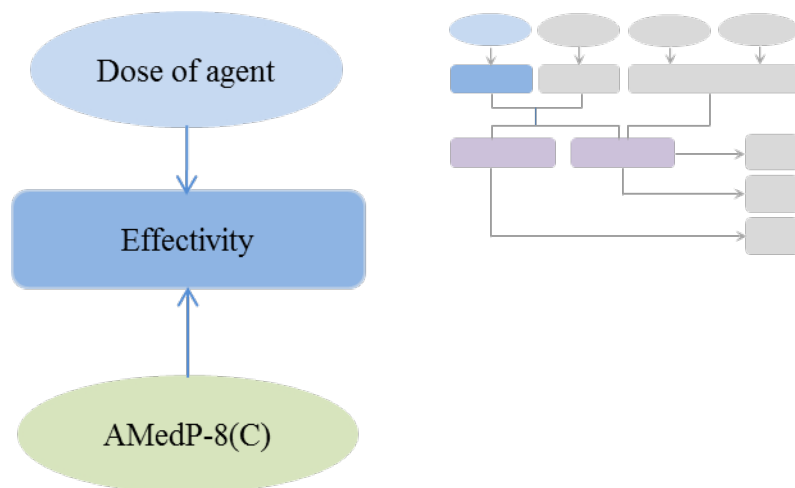
Figure 6. Modeling scheme for effectivity. The light blue oval indicates the user input, the dark blue rectangle indicates the modeling parameter calculations and the green oval indicates data used to establish this parameter. The inset image shows the parts of the larger Figure 1 modeling scheme that are influenced by this parameter.

Value or function:

Effectivity is modeled as a log-probit function with a median effective dose (ED_{50}) of 0.1 μg/man and a probit slope of 12.9.

Individuals for Whom this Parameter Applies:

The effectivity parameter is applied to all individuals, but may be modified by other parameters described below (like the "Vaccine Efficacy" parameters).

Rationale:

This parameter was taken from AMedP-8(C).[31]

Duration of the Latent Period

The latent period of disease is the time between exposure and the onset of symptoms. The distribution of the latent period duration following inhalation of botulinum neurotoxin is established by AMedP-8(C).[32] The schematic shown below (Figure 7) illustrates the data that influence this modeling parameter.

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[31] NATO. "AMedP-8(C) NATO Planning Guide for the Estimation of CBRN Casualties Ratification Draft 1." February 2010.
[32] NATO. "AMedP-8(C) NATO Planning Guide for the Estimation of CBRN Casualties Ratification Draft 1." February 2010.
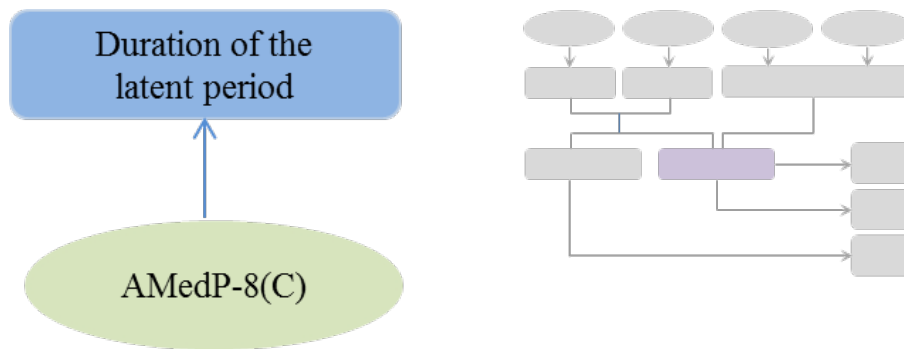
Figure 7. Modeling scheme for the duration of the latent period. The dark blue rectangle indicates the modeling parameter calculations and the green oval indicates data used to establish this parameter. The inset image shows the parts of the larger Figure 1 modeling scheme that are influenced by this parameter.

Value or function:

> The duration of the latent period is modeled with a minimum duration of one day, and otherwise follows a log-normal distribution with a median of one day and a standard deviation of 2.32 days.

Individuals for Whom this Parameter Applies:

> The "Duration of the Latent Period" parameter is applied to all symptomatic individuals.

Rationale:

> This parameter was taken from AMedP-8(C).[33]

Lethality

The "Lethality" parameter defines the likelihood of death in individuals with inhalational botulism. As established by AMedP-8(C),[34] the "Lethality" parameter operates as a function of dose where the likelihood of death increases with inhaled dose. The schematic shown below (Figure 8) illustrates the data that influence this modeling parameter.

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[33] NATO. "AMedP-8(C) NATO Planning Guide for the Estimation of CBRN Casualties Ratification Draft 1." February 2010.
[34] NATO. "AMedP-8(C) NATO Planning Guide for the Estimation of CBRN Casualties Ratification Draft 1." February 2010.
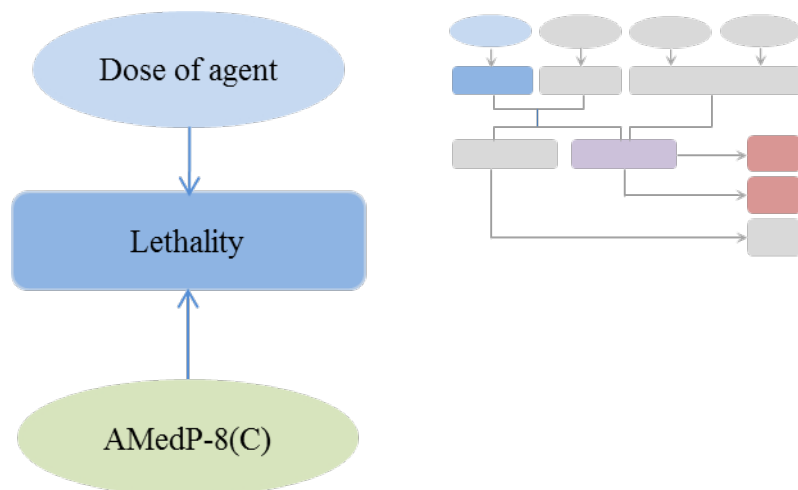
Figure 8. Modeling scheme for lethality. The light blue oval indicates the user input, the dark blue rectangle indicates the modeling parameter calculations and the green oval indicates data used to establish this parameter. The inset image shows the parts of the larger Figure 1 modeling scheme that are influenced by this parameter.

Value or function:

Lethality is modeled as a log-probit function with a median lethal dose (LD_{50}) of 0.8 µg/man and a probit slope of 12.9.

Individuals for Whom this Parameter Applies:

The lethality parameter is applied to all symptomatic individuals but may be modified by other parameters (like the antitoxin treatment parameters).

Rationale:

This parameter was taken from AMedP-8(C).[35]

Duration of the Symptomatic Period

The "Duration of the Symptomatic Period" parameters describe the length of each symptomatic stage of botulism. We use the term symptomatic period; this same period is referred to by AMedP-8(C) as the "duration of illness." The symptomatic period is divided into three stages.[36] Signs of Stage 1 include fatigue, drooping eyelids, and blurred or double vision and is described by AMedP-8(C) as severity level 2 (moderate). Stage 2 follows with more severe symptoms including acute symmetrical descending flaccid paralysis and progressive muscle weakness in the head and neck, and is described by AMedP-8(C) as severity level 3 (severe). Stage 3 takes on two forms: survivors experience a gradual reversal of muscle paralysis (severity level 2, moderate) while non-survivors experience paralysis of the respiratory muscles, respiratory failure, and death (severity level 4, very severe). The schematic shown below (Figure 9) illustrates the data that influence this modeling parameter.

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[35] NATO. "AMedP-8(C) NATO Planning Guide for the Estimation of CBRN Casualties Ratification Draft 1." February 2010.
[36] NATO. "AMedP-8(C) NATO Planning Guide for the Estimation of CBRN Casualties Ratification Draft 1." February 2010.
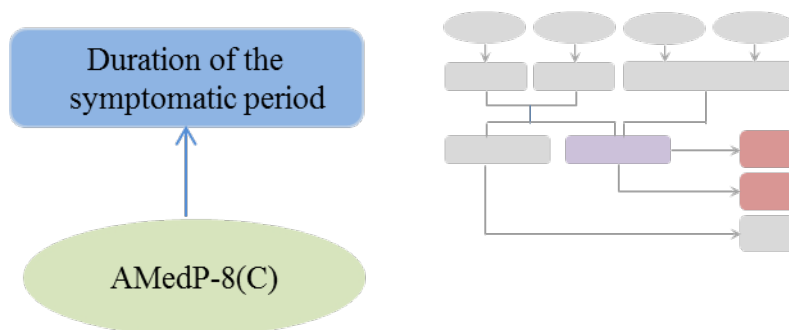
Figure 9. Modeling scheme for the duration of the symptomatic period. The dark blue rectangle indicates the modeling parameter calculations and the green oval indicates data used to establish this parameter. The inset image shows the parts of the larger Figure 1 modeling scheme that are influenced by this parameter.

Value or function:

Table 2. Duration of the Symptomatic Period Parameters		
	Survivor	**Non-survivor**
Duration of Stage 1	Normal distribution with a mean of 1 day and SD of 1.12 days	1/3 of the length of the symptomatic period, which is an exponential distribution where $\lambda = 0.318$
Duration of Stage 2	Normal distribution with a mean of 2 weeks and SD of 8.54 days	1/3 of the length of the symptomatic period, which is an exponential distribution where $\lambda = 0.318$
Duration of Stage 3	6 months	1/3 of the length of the symptomatic period, which is an exponential distribution where $\lambda = 0.318$

Individuals for Whom this Parameter Applies:

The "Duration of the Symptomatic Period" parameter is applied to all untreated, symptomatic individuals; the symptomatic period for treated individuals is described by other parameters below (the "Duration of Symptoms in Treated Survivors" and the "Duration of Symptoms in Treated Non-Survivors").

Rationale:

Survivor

The duration of the symptomatic period for each symptomatic stage in untreated survivors was taken from AMedP-8(C). However, the AMedP-8(C) parameters lack the variability necessary for our stochastic model, thus a standard deviation was added. As stated in the technical reference manual for AMedP-8(C), there are no available data on the duration of each symptomatic stage in untreated humans.[37] Standard deviations for each stage of the symptomatic period were therefore estimated from experimental animal studies, as described below.

Herrero et al. injected rhesus monkeys with botulinum neurotoxin and reported the onset and duration of four symptoms in individual untreated animals; nine animals in this study survived.

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[37] Curling et al. "Technical Reference Manual: NATO Planning Guide for the Estimation of Chemical, Biological, Radiological, and Nuclear (CBRN) Casualties, Allied Medical Publication-8(C)." August 2010.

Since AMedP-8(C) includes the occurrence of ptosis and muscular weakness as markers for Stages 1 and 2 (respectively) of botulism progression, data describing the onset and duration of these two symptoms in the untreated rhesus monkeys were used to determine mean durations of each stage.[38] The length of Stage 1 (the duration of time between the onset of ptosis and the onset of muscular weakness) was calculated to be 1.81 days with a standard deviation of 2.03 days (112% of the mean). The length of Stage 2 (the duration of time between the onset of muscular weakness and the initiation of recovery) was calculated to be 4.00 days with a standard deviation of 2.44 days (61% of the mean).[39] Details from this study are included in Table A-1, Appendix 1.

Since the mean durations for each symptomatic stage found in the monkey study are different from the durations established by AMedP-8(C), we defined the standard deviation in our model as a percentage of the mean duration of each stage. We therefore estimated the standard deviation in humans to be 1.12 days for Stage 1 (112% of 1 day), and 8.54 days for Stage 2 (61% of 14 days). We also assume that the duration of each stage is distributed normally.

Since AMedP-8(C) suggests an extremely long period for Stage 3 in untreated survivors (six months), we assume that no untreated survivor will be capable of returning to work in a timeline relevant to military operation (see the "Duration of Symptoms in Treated Survivors" section and the "Loss of Work" section). Therefore, it was not necessary for our model to calculate a standard deviation for this period.

Non-Survivor
The duration of each symptomatic stage in non-survivors was taken from AMedP-8(C), which describes the length of each symptomatic stage as being equal to 1/3 of the length of the symptomatic period (an exponential distribution where $\lambda = 0.318$).[40] Because the duration of each stage in non-survivors has inherent variability from the exponential distribution, there was no need to add a standard deviation to this parameter. Note that AMedP-8(C) does not set a minimum for the total duration of the symptomatic period; therefore it is possible for the model to report the symptomatic period duration as zero. A symptomatic period of zero indicates that death occurs on the same day that symptoms begin.

Time of Death

The "Time of Death" parameter describes the length of time between when an individual is exposed and the time that the individual dies. The time of death is established based on the duration of the latent period and the duration of the symptomatic period. The schematic shown below (Figure 10) illustrates the data that influence this modeling parameter.

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[38] Herrero BA et al. "Experimental Botulism in Monkeys – A Clinical Pathological Study." *Experimental and Molecular Pathology.* **6**(1). 1967.

[39] The initiation of recovery was defined as the day in which the animal first stopped displaying a previously observed symptom.

[40] NATO. "AMedP-8(C) NATO Planning Guide for the Estimation of CBRN Casualties Ratification Draft 1." February 2010.
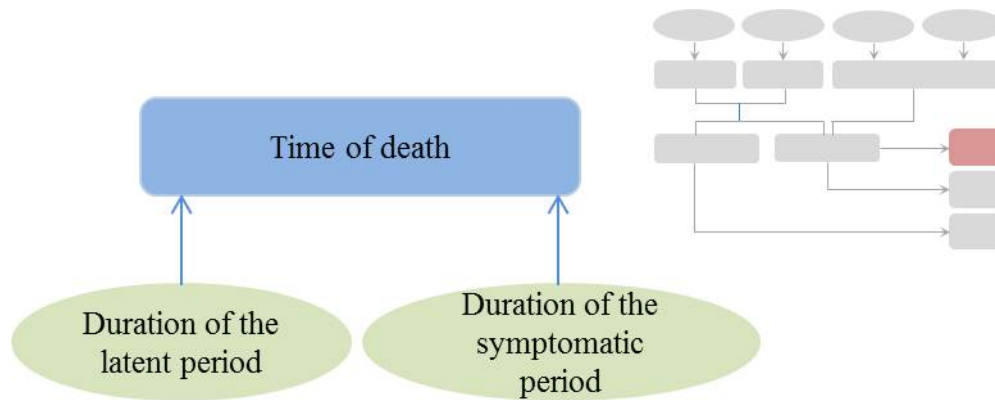
Figure 10. Modeling scheme for the time of death. The dark blue rectangle indicates the modeling parameter calculations and the green ovals indicate data used to establish this parameter. The inset image shows the parts of the larger Figure 1 modeling scheme that are influenced by this parameter.

Value or function:

> Time of death = Duration of the latent period + Duration of the symptomatic period

Individuals for Whom this Parameter Applies:

> The "Time of Death" parameter is applied to all individuals that die; any parameters that modify the duration of the latent period or the duration of the symptomatic period will also affect the time of death.

Rationale:

> The time that an individual dies is equal to the sum of the duration of the latent period and the duration of the symptomatic period. The untreated latent period and symptomatic periods are described in parameters above, and in some cases are modified by the countermeasure parameters described below.

Botulinum Toxoid Vaccine

Although the United States has no licensed vaccine for the prevention of botulism, until very recently the subcutaneous vaccine, Pentavalent (ABCDE) Botulinum Toxoid (PBT) was available as an investigational new drug for botulism researchers and at-risk military personnel. The vaccine was discontinued in November of 2011 due to declining immunogenicity and increased occurrence of moderate adverse reactions.[41] A number of vaccines in development have reached clinical trials in humans, but the vaccine parameter for this model was developed based on PBT because PBT efficacy data is publically available.[42] In addition, given that the vaccine was discontinued in 2011, it is likely that some members of the military are still protected by the PBT vaccine. If additional information on new vaccines becomes available, advanced users are able to alter most "Vaccine Efficacy" parameters on the advanced users tab.

Although there are no empirical human data on the efficacy of the vaccine, animal data suggest that PBT offers very good protection against inhaled type A botulinum neurotoxin, the toxin modeled by AMedP-

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[41] CDC. "Notice of CDC's Discontinuation of Investigational Pentavalent (ABCDE) Botulinum Toxoid Vaccine for Workers at Risk for Occupational Exposure to Botulinum Toxins." *Morbidity and Mortality Weekly Report.* October 28, 2011. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6042a3.htm. Accessed on December 15, 2011.

[42] Zygmunt F et al. "Botulinum Toxin." *Medical Aspects of Biological Warfare.* Department of Defense, Office of the Surgeon General, US Army, Borden Institute, Washington DC. 2007.

8(C). Below, we develop vaccine-related parameters for "Complete" and "Incomplete" vaccination. "Complete" vaccination includes four doses of vaccine at weeks 0, 2, 12 and 26, plus annual boosters, where the last booster (or dose) is received within four years of exposure.[43] "Incomplete" vaccination includes only the first three doses (the dosing regimen that was used until 2004.)[44,45] Though it is possible that vaccinated or partially vaccinated individuals who develop symptoms may have decreased severity or duration of disease, there is very little data available on the changes in the disease course after vaccination. For this reason, we assume that the population for which vaccination fails will experience the same disease course as the population without countermeasures applied (see the "Untreated Disease Course" section).

Vaccine Efficacy

The "Vaccine Efficacy" parameter defines the likelihood that vaccination will prevent symptom onset in individuals exposed to botulinum neurotoxin A, who would be expected to fall ill without vaccination. This parameter was established using human antibody titer data and experimental data from guinea pig and non-human primate challenge studies. The schematic shown below (Figure 11) illustrates the data that were used to establish the pre-exposure vaccine efficacy modeling parameter.
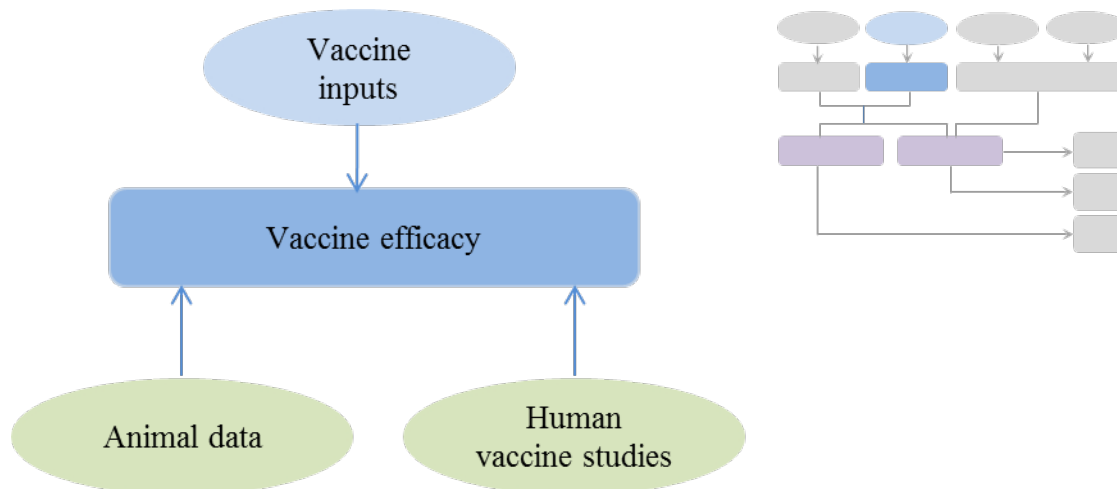

Figure 11. Modeling scheme for vaccine efficacy. The light blue oval indicates the user input, the dark blue rectangle indicates the modeling parameter calculations and the green ovals indicate data used to establish this parameter. The inset image shows the parts of the larger Figure 1 modeling scheme that are influenced by this parameter.

Value or Function:
        Incomplete vaccination:
        When: $W = 0$
                $V_{incomplete} = 0\%$ (equal to unvaccinated)

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[43]   We did not develop a modeling parameter for vaccine efficacy when individuals received the full vaccine course more than four years before exposure. Although these individuals are likely to have some protection, we found no data that could be used to support parameter development. Therefore, the modeling input page does not allow users to choose complete vaccination more than four years prior to exposure. Users who select complete vaccination (which assumes that the last dose or booster was within four years of exposure) as a proxy will likely underestimate the number of causalities, while users who select no vaccination as a proxy will likely overestimate casualties.

[44]   Smith LA and Rusnak JM. "Botulinum Neurotoxin Vaccines: Past, Present, and Future." *Critical Reviews in Immunology*. **27**(4). 2007.

[45]   Smith LA. "Botulism and vaccines for its prevention." *Vaccine*. **27**(Suppl 4). 2009.

When: $101.7 * e^{\left(-0.5 * \left(\frac{\ln\left(W/_{6.166}\right)}{1.068}\right)^2\right)} > 100$

$\qquad V_{incomplete} = 100\%$

Otherwise: $V_{incomplete} = 101.7 * e^{\left(-0.5 * \left(\frac{\ln\left(W/_{6.166}\right)}{1.068}\right)^2\right)}$

Where: $V_{incomplete}$ = the efficacy of vaccine at time *W* weeks.
$\qquad$ *W* = number of weeks after the third vaccine dose that exposure occurs.

Complete vaccination:
$V_{complete} = 98\%$

Where: $V_{complete}$ = the efficacy of vaccine after four or more doses plus annual boosters, where the last booster (or dose) is received within four years of exposure.

Individuals for Whom this Parameter Applies:
$\qquad$ In a vaccinated individual, the "Vaccine Efficacy" parameter modifies the probability that the individual will develop symptoms. Based on the individual's input dose, the probability of developing symptoms in the absence of vaccination is calculated (see the "Effectivity" parameter). The "Vaccine Efficacy" parameter, which reduces the probability of developing symptoms by the percentage described in the equations above, is then applied.

Rationale:
$\qquad$ The "Vaccine Efficacy" parameter establishes the efficacy of vaccine after three doses of the PBT vaccine (incomplete vaccination) or four doses of the PBT vaccine, with the last dose or booster received within four years of exposure (complete vaccination).[46] The effect of agent dose on vaccine efficacy and the animal and human studies that support this parameter are described below.

Vaccine Efficacy and Agent Dose

To establish the effect of agent dose on vaccine efficacy, we assessed antibody titers from vaccinated humans and challenge studies with vaccinated animals. Several human studies reported titers of antibodies against botulinum neurotoxin serotype A at various times post-vaccination. While some of the studies reported a specific titer for each individual studied, many reported only the percentage of individuals that had a measurable antibody titer, typically defined as 0.02 IU/mL or greater.[47]

Vaccinated monkeys and guinea pigs with antibody titers ranging from 0.01 to 0.25 IU/ml challenged via the respiratory route with toxin doses ranging from 2 to 100 LD_{50}s show no

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[46] While PBT was designed to protect against toxin subtypes A-E, our parameters were developed using only data for protection against subtype A, since our model assumes exposure to toxin subtype A. Although there are no experimental human data on the efficacy of the vaccine, animal data suggest that PBT offers very good protection against inhaled type A botulinum neurotoxin (as explained in the "Vaccine Efficacy" section of this document).

[47] IU= international unit, defined as "the amount of antibody neutralizing 10,000 mouse intraperitoneal 50% lethal doses of type A, B, C or D botulinum toxin or 1,000 mouse intraperitoneal 50% lethal doses of type E." The citation for this statement follows: *Siegel LS. "Human Immune Response to Botulinum Pentavalent (ABCDE) Toxoid Determined by a Neutralization Test and by an Enzyme-Linked Immunosorbent Assay." *Journal of Clinical Microbiology*. **26**(11). 1998.

significant difference in their level of protection (Figure 12a and b).[48,49] Based on these animal data, our model assumes that regardless of the antibody titer or dose of toxin, all individuals with measurable titers are equally well protected.
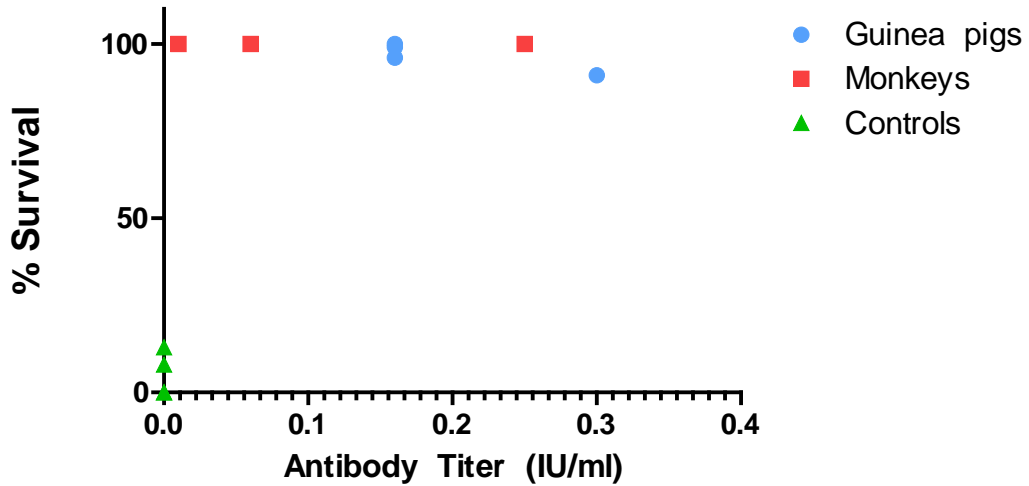
(a)
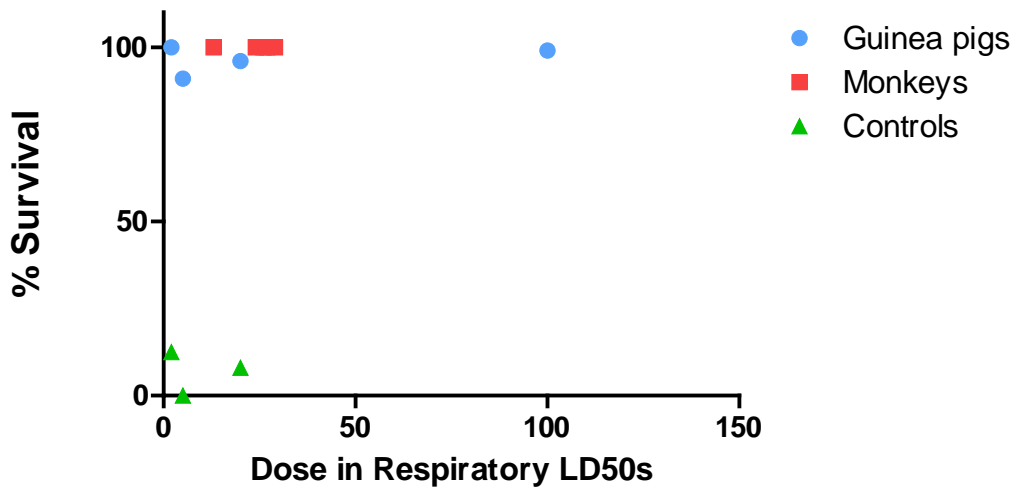
(b)

Figure 12. Percent survival as a function of (a) serum antibody titer and (b) dose of toxin in guinea pigs (blue circles) and monkeys (red squares). The monkey study did not provide information on controls; therefore, the control groups include only unvaccinated guinea pigs.

Excluded Data

Data from one similar study were excluded from the analysis displayed in Figure 12 above.[50] This study suggests that protection from the toxin changes as a function of antibody titer,

48 Smith LA and Rusnak JM. "Botulinum Neurotoxin Vaccines: Past, Present, and Future." *Critical Reviews in Immunology*. **27**(4). 2007.
49 Cardella MA et al. "Resistance of Guinea Pigs Immunized with Botulinum Toxoids to Aerogenic Challenge with Toxin." US Army Biological Laboratories, Fort Detrick Maryland. Technical Manuscript 66. May 1963.

meaning that higher titers of antibody are needed to protect from larger doses of toxin. However, the doses of toxin used in the study ($1.8 \times 10^5 - 5.0 \times 10^6$ mouse intraperitoneal LD_{50}s) were much higher than those in the other studies included, and the quantity of toxin administered would be unrealistic in an aerosolized attack. Thus, data from this study were not included in formulating the "Vaccine Efficacy" parameter.

Efficacy of Incomplete Vaccination

Since the fourth dose of the PBT was has not always been part of the vaccination regimen,[51,52] we established a "Vaccine Efficacy" parameter for incomplete vaccination (three doses of vaccine) in addition to a parameter for complete vaccination (four doses of vaccine with the last booster or dose received within four years of exposure). Four human studies of antibody titers post-vaccination were conducted using the pentavalent vaccine before the fourth dose was added to the recommended vaccination regimen. All four studies showed that the majority of participants had a measurable antibody[53] titer soon after the third dose, but the number of individuals with a measurable titer decreased drastically in the months following vaccination. These studies are briefly summarized below.

In 1963 Fiock et al.[54] published an article that reported on the antibody titers of 17 individuals vaccinated with three doses of the pentavalent vaccine. Antibody titers greater than or equal to 0.02 IU/ml were seen in 65% of individuals two weeks after the third dose, but titers dropped to undetectable levels in all of the individuals by 42 weeks after the third dose of the vaccine. A 1988 study by Siegel[55] found that antibody titers greater than or equal to 0.08 IU/ml could be detected in 88% of volunteers approximately two weeks (14 days $\pm$ 2 days)[56] after vaccination[57], but could be detected in only 52% of volunteers 40 weeks later. In the late 1990s and early 2000s, a third study was performed using serum collected from USAMRIID laboratory workers.[58] This study found that 92% of the tested individuals had antibody titers of at least 0.02 IU/ml four weeks after the third dose of the vaccine, but only 33% of individuals had detectable antibody levels six to twelve months after the third dose. Finally, the Joint Vaccine Acquisitions Program (JVAP) sponsored a fourth study which demonstrated that only 30.8% of individuals vaccinated using the three dose regimen had an antibody subtype A titer of at least 0.2 IU/ml at month six. The four studies described above suggest that antibody levels (and likely, protection against botulinum neurotoxin) decrease over time following three doses of vaccine. The results of the

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[50] United States Army Biological Laboratories, Fort Detrick. "Technical Manuscript 141: Botulinum Toxoids." 443673. June 1964.

[51] Smith LA and Rusnak JM. "Botulinum Neurotoxin Vaccines: Past, Present, and Future." *Critical Reviews in Immunology.* **27**(4). 2007.

[52] Smith LA. "Botulism and vaccines for its prevention." *Vaccine.* **27**(Suppl 4). 2009.

[53] Note that a measurable titer varied from study to study and ranged from 0.02 IU/ml to 0.2 IU/ml.

[54] Fiock MA et al., "Studies of Immunity to Toxins of Clostridium Botulinum. IX. Immunologic Response of Man to Purified Pentavalent ABCDE Botulinum Toxoid." *The Journal of Immunology.* **90**(5). 1962.

[55] Siegel LS. "Human Immune Response to Botulinum Pentavalent (ABCDE) Toxoid Determined by a Neutralization Test and by an Enzyme-Linked Immunosorbent Assay." *Journal of Clinical Microbiology.* **26**(11). 1988.

[56] The individuals included in our analysis had blood drawn for antibody testing on days 13, 14, 15 and 16 after the last immunization (Siegel 1988 Table 1). No individuals had blood drawn on day 11 (14-3) or day 17 (14+3), therefore to be more precise we state that antibody titers were 14 $\pm$ 2 days. Please note that individuals tested on days 18, 20, 21, and 22 were excluded from this analysis.

[57] Table 1 on page 2353 of Siegel (1988) reports two individuals with a neutralization titer of <0.08 IU/ml and one with a neutralization titer of 0.07 IU/ml. We assume that 0.07 IU/ml is a typo and should be 0.08 IU/ml, since it appears the level of detection is 0.08 IU/ml.

[58] Smith LA and Rusnak JM. "Botulinum Neurotoxin Vaccines: Past, Present, and Future." *Critical Reviews in Immunology.* **27**(4). 2007.

JVAP study prompted the addition of a fourth dose of PBT to the vaccine regimen at month six.[59,60]

Given that the minimum titer reported varied between studies, our modeling parameter was established using only data from the Fiock and the Smith and Rusnak studies. Both reported a minimum titer of 0.02 IU/ml which, according to Fiock, is enough antibody to neutralize 30 LD_{50}s.

These two studies were used to establish modeling parameters for individuals who receive just three doses of the vaccine before any additional boosts, which we define as "incomplete" vaccination. The graph below (Figure 13), shows human antibody titer data in individuals who had not received a booster at one year after the first dose. The Gaussian curve fit to these data is physiologically relevant — it mirrors the expected rise and fall of antibody titer as the immune response is mounted, peaks, and wanes.
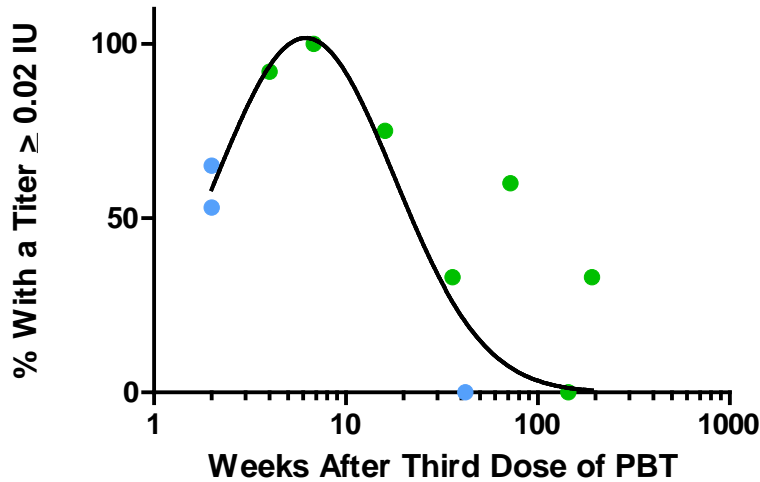

Figure 13. Efficacy of incomplete vaccination. Data from the Fiock (blue) and Smith and Rusnak (green), studies indicating the percent of individuals with a botulinum antibody subtype A titer ≥ 0.02 IU at various time points after the third dose of PBT. Note that the data points are weighted to reflect the number of individuals represented by each point. The equation of the Gaussian curve is

$$V_{incomplete} = 101.7 * e^{\left(-0.5 * \left(\frac{\ln(W/6.166)}{1.068}\right)^2\right)}$$ **where** $V_{incomplete}$ **= the efficacy of vaccine after three doses of vaccine** W **weeks after the third dose.**

Since there are no data on the efficacy of fewer than three doses of vaccine and because the first three doses occur over a short period of time (increasing the likelihood of compliance and decreasing the likelihood that exposure would occur between doses), we do not consider the efficacy of less than three doses of vaccine. The efficacy of incomplete vaccination (three doses)

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[59] Centers for Disease Control. "IND Protocol: Use of a Pentavalent (ABCDE) Botulinum Toxoid Aluminum Phosphate Adsorbed (PBT) for Workers at Risk of Occupational Exposure to Botulinum Toxins." BB-IND 161 Protocol CDC IRB #392, Version 8.0.

[60] Battelle Memorial institute, Chemical Warfare/Chemical and Biological Defense Information Analysis Center. "Evaluation of Safety and Immunogenicity of Pentavalent Botulinum Toxoid (A-E) Administered to Healthy Volunteers – Continuation of Study for Determination of Booster Vaccination Interval." Contract No. SPO900–94–D–0002, Task No. 161, Delivery Order 0088. November 2002.

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can be described by the equation of the line in Figure 13 above: $V_{incomplete} = 101.7 *$ $e^{(-0.5 * (\frac{\ln(W/6.166)}{1.068})^2)}$.

Efficacy of Complete Vaccination

While the immune response following vaccination with three doses of PBT wanes by six months after the first dose, the addition of a booster leads to significantly better protection. The Joint Vaccine Acquisitions Program sponsored study, which showed that only about 30% of individuals had a measurable titer at six months,[61,62] prompted the addition of a fourth dose at 26 weeks in 2004.[63,64] In three of the vaccine studies described in the previous section, a fourth "booster" dose was given at 52 weeks, which we used to approximate the effect of the recommended fourth vaccine dose.

Fiock et al. found that eight weeks after a 52 week booster, 100% of individuals had a measurable botulinum neurotoxin antibody titer. [65] In the study by Siegel et al., 100% of volunteers who were boosted at week 52 had a detectable antibody titer two weeks later. [66] In the study by Smith and Rusnak, of the individuals who were boosted at week 52, 100% had detectable levels of antibody 21 to 35 days after the boost, and 95% maintained detectable levels for more than four years with no further boosting.[67] One other source, an abstract "Immunogenicity of Pentavalent Botulinum Toxoid Vaccine" authored by Rusnak et al., states that 95% of individuals have an "adequate" titer 30 to 180 days after the last booster.[68]

Approximately 98% (between 95% and 100%) of participants that were checked for an antibody titer between one month and four years after receiving a boost at 52 weeks had a measurable titer. We estimate that nearly all individuals who receive the complete vaccination regimen will be well protected. Thus, our model predicts that 98% of individuals who receive four or more doses of the vaccine would be protected ($V_{complete} = 98\%$) if the vaccine was administered within four years of exposure.

Given the lack of data, we did not develop a modeling parameter for vaccine efficacy when individuals received the full vaccine course more than four years before exposure. Although these individuals are likely to have some protection, we found no data that could be used to support parameter development. Therefore, the modeling input page does not allow users to choose complete vaccination more than four years prior to exposure. Users who select complete

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[61] Centers for Disease Control. "IND Protocol: Use of a Pentavalent (ABCDE) Botulinum Toxoid Aluminum Phosphate Adsorbed (PBT) for Workers at Risk of Occupational Exposure to Botulinum Toxins." BB-IND 161 Protocol CDC IRB #392, Version 8.0.

[62] Battelle Memorial institute, Chemical Warfare/Chemical and Biological Defense Information Analysis Center. "Evaluation of Safety and Immunogenicity of Pentavalent Botulinum Toxoid (A-E) Administered to Healthy Volunteers – Continuation of Study for Determination of Booster Vaccination Interval." Contract No. SPO900–94–D–0002, Task No. 161, Delivery Order 0088. November 2002.

[63] Smith LA and Rusnak JM. "Botulinum Neurotoxin Vaccines: Past, Present, and Future." *Critical Reviews in Immunology.* **27**(4). 2007.

[64] Smith LA. "Botulism and vaccines for its prevention." *Vaccine.* **27**(Suppl 4). 2009.

[65] Fiock MA et al., "Studies of Immunity to Toxins of Clostridium Botulinum. IX. Immunologic Response of Man to Purified Pentavalent ABCDE Botulinum Toxoid." *The Journal of Immunology.* **90**(5). 1962.

[66] Siegel LS. "Human Immune Response to Botulinum Pentavalent (ABCDE) Toxoid Determined by a Neutralization Test and by an Enzyme-Linked Immunosorbent Assay." *Journal of Clinical Microbiology.* **26**(11). 1988.

[67] Smith LA and Rusnak JM. "Botulinum Neurotoxin Vaccines: Past, Present, and Future." *Critical Reviews in Immunology.* **27**(4). 2007.

[68] Rusnak J et al. "Immunogenicity of Pentavalent Botulinum Toxoid Vaccine." Abstract, available at: http://www.acponline.org/about_acp/chapters/usaf/infectious_disease2.pdf. Accessed June 4, 2012.

vaccination (which assumes that the last dose or booster was received within four years of exposure) as a proxy will likely underestimate the number of causalities, while users who select no vaccination as a proxy will likely overestimate casualties.

Botulism Treatment: Antitoxin and Mechanical Ventilation

Antitoxin

The investigational heptavalent botulinum antitoxin (HBAT) recently became the primary antitoxin available for non-infant cases of botulism in the United States.[69,70] HBAT, an equine antitoxin, targets BoNT serotypes A, B, C, D, E, F and G and was recently supplied to the Strategic National Stockpile.[71]

Available under an Investigational New Drug (IND) protocol, HBAT is the current standard for treatment of botulism and has been administered in at least one human clinical case; however, there are no publicly available efficacy data on the investigational new drug.[72,73] For this reason, the efficacy of HBAT was modeled based on the efficacies of previously available antitoxins. Two FDA-approved antitoxins have been used in the recent past: trivalent equine antitoxin (which targets BoNT serotypes A, B and E) and bivalent equine antitoxin (which targets BoNT A and B).[74,75] One other antitoxin derived from human serum is currently available, but is used exclusively in infants and was therefore excluded from this analysis.[76,77]

Evidence suggests that HBAT antitoxin treatment is sufficient to neutralize any dose of circulating toxin that could be reasonably expected from an aerosol exposure; information on the neutralization capabilities of HBAT is included in Appendix 2. Given this evidence, our model does not consider the dose of agent or the dose of antitoxin when considering antitoxin efficacy.

Although HBAT antitoxins are considered treatment, they only act on circulating BoNT that is not bound to nerve endings, most likely by blocking or inhibiting the toxin. Therefore, antitoxin does not reverse

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[69] Fagan RP et al. "Initial recovery and rebound of Type F intestinal colonization botulism after administration of investigational heptavalent botulinum antitoxin." *Clinical Infectious Diseases.* E-publication ahead of print. September 6, 2011.

[70] CDC. "Investigational Heptavalent Botulinum Antitoxin (HBAT) to Replace Licensed Botulinum Antitoxin AB and Investigational Botulinum Antitoxin E." *Morbidity and Mortality Weekly Report (MMWR).* **59**(10). March 19, 2010. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5910a4.htm. Accessed on October 21, 2011.

[71] Cangene. "Products: Biodefense Hyperimmunes." February 22, 2011. http://www.cangene.com/products_hyperimmunes.shtml#Botulism. Accessed on October 21, 2011.

[72] Fagan RP et al. "Initial recovery and rebound of Type F intestinal colonization botulism after administration of investigational heptavalent botulinum antitoxin." *Clinical Infectious Diseases.* E-publication ahead of print. September 6, 2011.

[73] Fagan R. Personal communication, September 8, 2011.

[74] Thanongsaksrikul, J and Chaicumpa W. "Botulinum Neurotoxins and Botulism: A Novel Therapeutic Approach." *Toxins.* 3. 2011.

[75] Zhang JC, Sun L, and Nie QH. "Botulism, where are we now?" *Clinical Toxicology.* **48**. 2010.

[76] The FDA has also approved a human botulism immune globulin (derived from human serum) for treatment of BoNT serotypes A, B, C, D and E, but this antitoxin is limited to suspected cases of infant botulism. Citations for these statements follow: *Thanongsaksrikul, J and Chaicumpa W. "Botulinum Neurotoxins and Botulism: A Novel Therapeutic Approach." *Toxins.* 3. 2011. *Arnon SS et al. "Botulism toxin as a biological weapon: medical and public health management." *Journal of the American Medical Association.* **285**(8). 2001.

[77] A number of other antitoxins are under development and in human clinical trials, but HBAT is the most readily available antitoxin in an emergency. Citation for this statement follows: *Zygmunt F et al. "Botulinum Toxin." *Medical Aspects of Biological Warfare.* Department of Defense, Office of the Surgeon General, US Army, Borden Institute, Washington DC. 2007.

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paralysis; it only prevents the progression of the disease.[78] Because botulism can lead to paralysis of muscles used for swallowing and breathing, treatment often requires supportive care; our analysis of antitoxin efficacy assumes that supportive care is provided as needed. This includes the use of medical supplies and equipment available in a hospital, such as feeding tubes and intravenous fluids. Mechanical ventilation, however, is considered separately, as it could be a limiting factor in treatment capacity.

Mechanical Ventilation

Because HBAT is only able to prevent the progression of botulism, some treated individuals may still be affected by respiratory paralysis and require mechanical ventilation, while other treated individuals will recover without ventilator support. It is therefore prudent to determine the probability of respiratory paralysis (and thus the need for mechanical ventilators) in individuals exposed to the toxin, as well as the efficacy of treatment with and without mechanical ventilation.

The use of mechanical ventilation in the absence of antitoxin treatment is not considered in our model because the data available in the literature are insufficient to establish the efficacy of ventilation alone. A limited number of reports describe treatment of botulism in human patients solely with mechanical ventilation, but it is not clear if any of these cases were caused by type A neurotoxin.[79,80,81]

Data from non-human primates do little to elucidate the efficacy of ventilation alone. One study of mechanical ventilation resulted in 100% mortality with or without antitoxin, despite the fact that similar experiments in the same studies showed a decrease in mortality when primates were treated with antitoxin alone.[82] Mechanical ventilation is not likely to increase mortality rates, as suggested by the data cited above. Given the 100% mortality rate in animals on ventilator support, it is likely that the ventilators were either malfunctioning or improperly fitted to the animals; therefore the report was excluded from our treatment analysis.

The sections below describe the efficacy of antitoxin treatment when mechanical ventilators are available, the probability of respiratory paralysis, and the efficacy of treatment when ventilator support is unavailable.

Efficacy of Antitoxin with Mechanical Ventilation if Needed

The "Efficacy of Antitoxin with Mechanical Ventilation if Needed" parameter establishes the reduction in the likelihood of death in individuals treated with antitoxin who have mechanical ventilation available if needed. This parameter was established using human clinical data and non-human primate experimental data. The schematic shown below (Figure 14) illustrates the data that were used to establish the "Efficacy of Antitoxin with Mechanical Ventilation if Needed" modeling parameter.

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[78] Dembek ZF. "Chapter 16 Botulinum Toxin" *Medical Aspects of Biological Warfare*. Office of the Surgeon General, United States Army, Falls Church, Virginia. Borden Institute Walter Reed Army Medical Center, Washington, DC. 2007.

[79] Glancy JAR. "Botulism- A Clinical Study of an Outbreak in the Yukon." *Canadian Medical Association Journal.* **10**(11). 1920.

[80] Sumi WT. "Botulism Treated with Tracheotomy and Respirator." *California Medicine*. **81**(4). 1954.

[81] Hurwitz S et al. "Botulism: A Case Report." *South African Medical Journal.* **57**(24). 1980.

[82] Oberst FW et al. "Evaluation of botulinum antitoxin, supportive therapy, and artificial respiration in monkeys with experimental botulism." *Clinical Pharmacology and Therapeutics.* **9**(2). 1968.
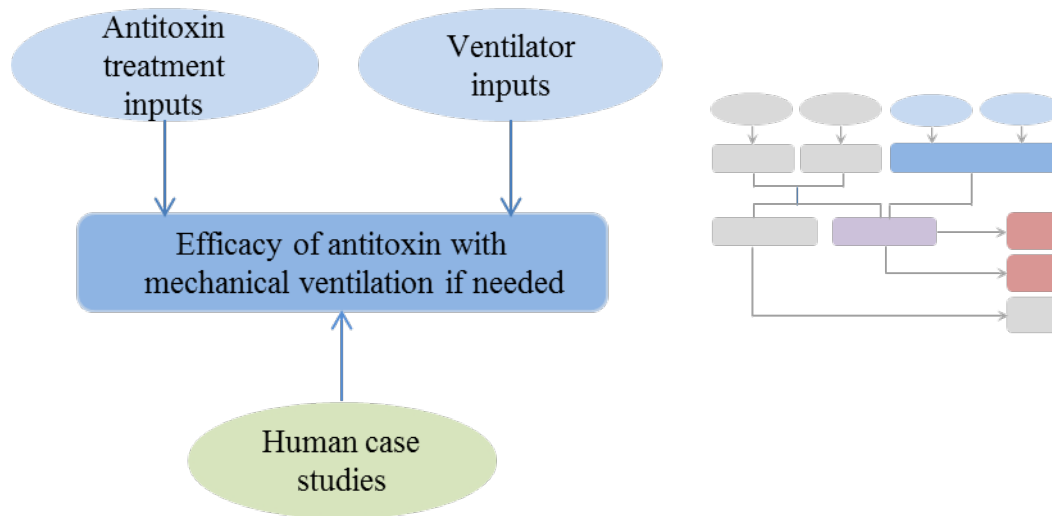
Figure 14. Modeling scheme for the efficacy of antitoxin with mechanical ventilation if needed. The light blue ovals indicate the user inputs, the dark blue rectangle indicates the modeling parameter calculations and the green oval indicates data used to establish this parameter. The inset image shows the parts of the larger Figure 1 modeling scheme that are influenced by this parameter.

Value or Function:

Table 3. Efficacy of Antitoxin with Mechanical Ventilation if Needed, Given as Percent Reduction in Number of Deaths as Compared to the Untreated Lethality Rate	
When antitoxin is administered on the same day as symptom onset (when the day of symptom onset is not the day of death)	78% effective
When antitoxin is administered one day after symptom onset (when one day after symptom onset is not the day of death)	72.5% effective
When antitoxin is administered two or more days after symptom onset (when the day of administration is not the day of death)	67% effective
When antitoxin is administered on the day of death*	33.5% effective
Efficacy is always 33.5% on the day of death, regardless of the length of the symptomatic period.	

Individuals for Whom this Parameter Applies:

In a symptomatic, treated individual the "Efficacy of Antitoxin with Mechanical Ventilation if Needed" parameter modifies the probability that the individual will die. For symptomatic, treated individuals the probability of death is first calculated as if the individual were not given mechanical ventilation or antitoxin (see the "Lethality" parameter). If the outcome is survival, the individual does not require mechanical ventilation. If the outcome is death, the "Efficacy of Antitoxin with Mechanical Ventilation if Needed" parameter is then applied, reducing the probability of death by the percentages described above. Some individuals will be saved by the addition of mechanical ventilation while others will still perish.

Rationale:

In this parameter, time of treatment administration is defined as the first time antitoxin treatment is initiated. Antitoxin is generally given in one dose at a single time point; however, mechanical ventilation and supportive care would be initiated as needed and continue until no longer necessary.

The information available on inhaled botulinum neurotoxin in humans is limited to one report. Published in 1962, this report describes three laboratory workers who contracted inhalational botulism following postmortem examination of laboratory animals who had succumbed to the disease. The workers were hospitalized three days after exposure. Each experienced symptoms similar to those of individuals with food-borne botulism (i.e. difficulty swallowing, indistinct speech and retarded extraocular motions). Antitoxin was administered to the patients on the fourth and fifth days after exposure and all three patients recovered.[83,84] It is difficult to establish the efficacy of antitoxin using these data since we do not know whether or not the individuals would have survived without antitoxin treatment. The disease these individuals experienced was similar to that seen following ingestion of the toxin; thus data from human cases of food-borne botulism were used to help establish the efficacy of antitoxin treatment.

Tacket et al. conducted a retrospective study of 132 cases of type A food-borne botulism.[85] Their data indicate that even when antitoxin and mechanical ventilation are available, deaths can still occur from cardiac arrest or other organ failure. Table 4 shows the survival rates of individuals given no antitoxin, those given antitoxin early (within the first 24 hours after symptom onset), and those given antitoxin late (more than 24 hours after symptom onset). Given that some individuals recovered without antitoxin treatment, the primary factor in determining treatment efficacy in this study is the improvement in rate of survival of treated versus untreated individuals. Treatment efficacy is presented in Table 4 as the percent reduction in number of deaths in the groups receiving antitoxin as compared to the untreated group. Tacket et al. state that some of the patients were put on ventilators, thus we assume that the treated individuals included in the study had ventilation available as needed, and are applicable to our "Antitoxin with Mechanical Ventilation" parameter.

Table 4. Survival Rates in Treated and Untreated Individuals			
	No Antitoxin	**Early Treatment**	**Late Treatment**
Survival [86]	54%	90%	85%
Efficacy of treatment	0% effective	78% effective	67% effective

While the human clinical data provide a good starting point for developing an antitoxin efficacy parameter, the data do not addresses whether antitoxin is efficacious when administered very close to the time of death. Tacket et al indicate that botulinum neurotoxin has been detected in

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83 Holzer E. "Botulism Caused by Inhalation." *MedizinischeKlinik*. 41. 1962 (German language version), referenced in Dembek et al. "Botulism: Cause, Effects, Diagnosis, Clinical and Laboratory Identification, and Treatment Modalities." *Disaster Medicine and Public Health Preparedness*. **1**(2). 2007.

84 Holzer E. "Botulism Caused by Inhalation." *MedizinischeKlinik*. 41. 1962. Translated by Edward Laohowicz, Medical-Legal Foundation Inc.

85 Tacket CO et al. "Equine Antitoxin Use and Other Factors that Predict Outcome in Type A Foodborne Botulism." *The American Journal of Medicine*.**76**. 1984.

86 Tacket CO et al. "Equine Antitoxin Use and Other Factors that Predict Outcome in Type A Foodborne Botulism." *The American Journal of Medicine*.**76**. 1984.

patient serum up to three and a half weeks after toxin ingestion.[87] Given that antitoxin works by binding unbound neurotoxin that is in the blood, antitoxin may retain some efficacy even late in the disease course. In the absence of additional data, our model assumes a 50% decrease in efficacy of treatment on the day of death.

These human data indicate that when compared to untreated individuals, the chance of death is reduced by 78% in those who receive antitoxin and mechanical ventilation within 24 hours of developing symptoms; thus, the efficacy of treatment on the day of symptom onset (treatment time = 0) was set at 78%. When treatment is given more than 24 hours after symptom onset, the data suggests that the efficacy rate falls to 67%. Depending on exactly when during the day symptom onset occurred, some individuals that receive treatment one day after symptom onset (treatment time = 1) will receive treatment within the first 24 hours of symptom onset, while others will have already passed this threshold, thus the efficacy of treatment one day after symptom onset was set to 72.5% (halfway between 78% and 67%). As previously noted, treatment given more than 24 hours after symptoms onset has an efficacy rate of 67%; thus we use this efficacy as our treatment parameter in those given treatment two or more days after symptom onset. The efficacy of treatment in individuals who receive treatment on the day of death was set at 33.5% (half of 67%), based the assumption of a 50% decrease in efficacy on the day of death. The day of death efficacy is applicable regardless of the length of the disease course.

Probability of Respiratory Paralysis

Our model reports respiratory paralysis in both treated and untreated patients. In treated patients, mechanical ventilators can be used to assist the breathing of individuals who suffer from respiratory paralysis due to botulism; however, not all patients will require ventilators to survive the disease. Since the parameter for "Efficacy of Antitoxin with Mechanical Ventilation if Needed" includes the use of ventilators when needed, the efficacy of treatment is expected to be reduced if mechanical ventilators are not available (see "Efficacy of Antitoxin Alone (Mechanical Ventilation Unavailable)"). The schematic shown below (Figure 15) illustrates the data that were used to establish the frequency of respiratory paralysis.
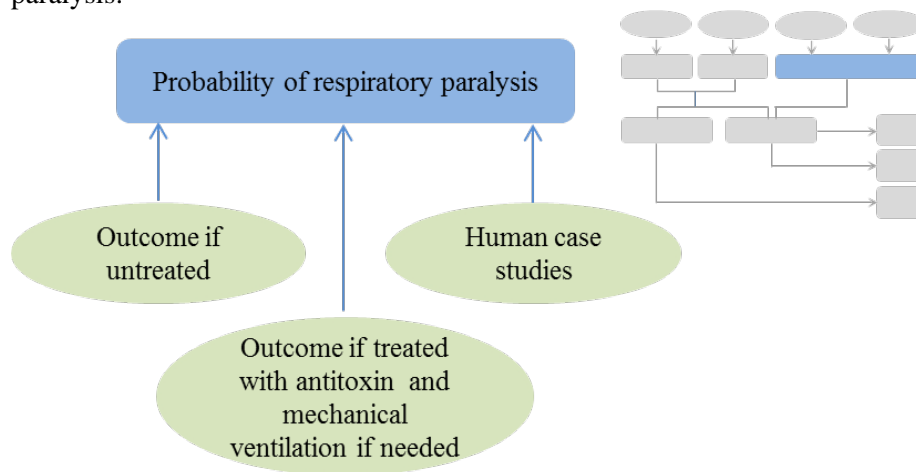

Figure 15. Modeling scheme for the probability of respiratory paralysis. The dark blue rectangle indicates the modeling parameter calculations and the green ovals indicate data used to establish this parameter. The inset image shows the parts of the larger Figure 1 modeling scheme that are influenced by this parameter.

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[87]   Tacket CO et al. "Equine Antitoxin Use and Other Factors that Predict Outcome in Type A Foodborne Botulism." *The American Journal of Medicine*. **76**. 1984.

Value or Function:

The "Probability of Respiratory Paralysis" parameter is calculated by first determining what an individual's outcome would be when untreated and what their outcome would be if antitoxin and ventilation were available.

If an individual would recover if untreated:
Probability of respiratory paralysis = 0%

If an individual would die even if all treatments (antitoxin, supportive care, and ventilation) were available:
Probability of respiratory paralysis = 100%

If an individual would die if left untreated but would survive if all treatments (antitoxin, supportive care, and ventilation) were available:
Probability of respiratory paralysis = 50%

Individuals for Whom this Parameter Applies:

For all symptomatic individuals, the model reports whether or not an individual experienced respiratory paralysis as an output.

Rationale:

Given that the cause of most botulism related deaths are due to respiratory failure, we assume that respiratory paralysis is ubiquitous in individuals who die. Likewise, we assume that respiratory paralysis does *not* occur in untreated survivors, since without treatment respiratory paralysis will quickly result in death. [88,89]

For those individuals who survive when given all treatments (antitoxin plus supportive care, and if necessary mechanical ventilation), some, but not all, may experience respiratory paralysis. We compiled data from more than 200 patients treated for Type A food-borne botulism, and found that mechanical ventilation was required for treatment in just over half of the cases. [90,91,92,93,94,95] Details of these studies are included in Table A-2, Appendix 2. [96] Our model

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[88] Treated individuals sometimes die from causes other than respiratory failure (for example cardiac arrest, ventilator-related infection, or other organ failure). However, in the absence of mechanical ventilation, these individuals would have most likely died of respiratory failure before the onset of these other causes of death. Therefore, our model assumes that 100% of treated individuals who die experience respiratory paralysis. Tacket CO et al. "Equine antitoxin use and other factors that predict outcome in type A foodborne botulism." *American Journal of Medicine.***76**(794-798). 1984.

[89] Arnon SS et al. "Botulinum toxin as a biological weapon." *Journal of the American Medical Association.* **285**(1059). 2001.

[90] Hatheway CH et al. "Antitoxin levels in botulism patients treated with trivalent equine botulism antitoxin to toxin types A, B, and E." *Journal of Infectious Diseases.* **150**(3). 1984.

[91] Kalluri P et al. "An outbreak of foodborne botulism associated with food sold at a salvage store in Texas." *Clinical Infectious Diseases.* **37**(11). 2003

[92] King LA. "Two severe cases of botulism associated with industrially produced chicken enchiladas, France, August 2008." *Eurosurveillance.* **13**(37). 2008.

[93] MacDonald KL et al. "Type A Botulism from Sautéed Onions. Clinical and Epidemiological Observations." *Journal of the American Medical Association.* **253**(9). 1985.

[94] Mann JM et al. "Patient Recovery from Type A Botulism: Morbidity Assessment following a Large Outbreak." *American Journal of Public Health.* **71**(3). 1981.

[95] Woodruff BA et al. "Clinical and laboratory comparison of botulism from toxin types A, B, and E in the United States, 1975-1988." *Journal of Infectious Diseases.* **166**(6). 1992.

[96] Although not explicitly stated, in these studies we assume that those individuals that received mechanical ventilation also received antitoxin, since antitoxin is the standard of care for hospitalized patients. Additionally, although a small percentage of the patients in these studies died, we had to ignore this mortality rate because the reports made no distinction between ventilator use in survivors and non-survivors. The details of each study in our analysis are included in Appendix 2.

therefore assumes that 50% of survivors who have both antitoxin and mechanical ventilation available will experience respiratory paralysis.

Efficacy of Antitoxin Alone (Mechanical Ventilation Unavailable)

The parameter for "Efficacy of Antitoxin Alone (Mechanical Ventilation Unavailable)" reduces the chance of death in individuals treated with antitoxin when mechanical ventilation is not available. This parameter was determined by applying an individuals' probability of respiratory paralysis to the chance of death if the individual had received all necessary treatment, as determined by the "Efficacy of Antitoxin with Mechanical Ventilation if Needed" parameter. The schematic shown below (Figure 16) illustrates the data that were used to establish the parameter for the efficacy of antitoxin treatment without mechanical ventilation.
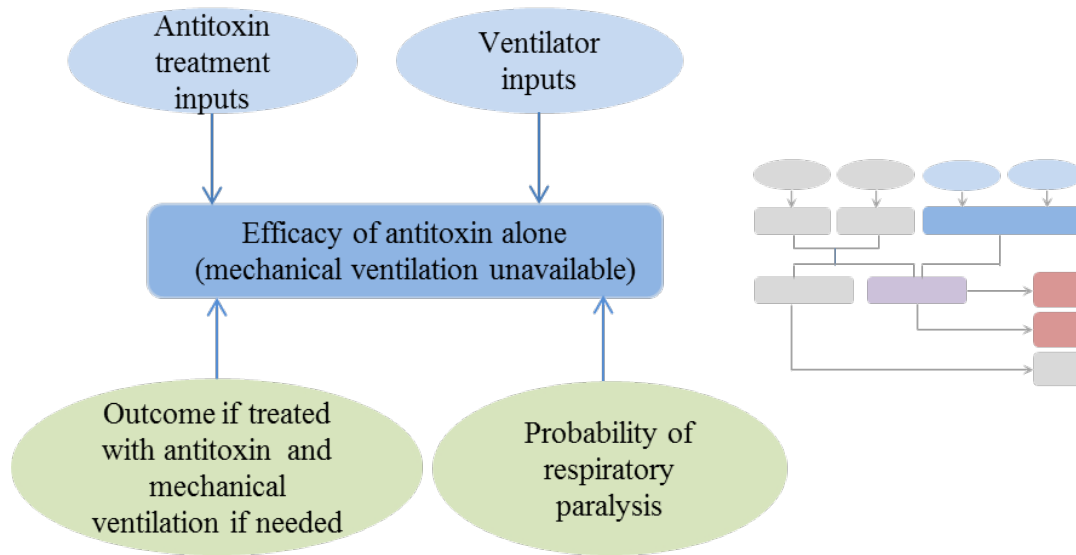

Figure 16. Modeling scheme for the efficacy of antitoxin alone (with mechanical ventilation unavailable). The light blue ovals indicate the user inputs, the dark blue rectangle indicates the modeling parameter calculations and the green ovals indicate data used to establish this parameter. The inset image shows the parts of the larger Figure 1 modeling scheme that are influenced by this parameter.

Value or Function:
>   If a treated patient experiences respiratory paralysis and mechanical ventilation is not available:
>>   Mortality = 100%
>   If a treated patient does not experience respiratory paralysis and mechanical ventilation is not available:
>>   Mortality = 0%

Individuals for Whom this Parameter Applies:
>   The "Efficacy of Antitoxin Alone (Mechanical Ventilation Unavailable)" parameter modifies the probability that a symptomatic individual treated with antitoxin alone will die. For each individual, the probability of death is first calculated as described in the "Efficacy of Antitoxin with Mechanical Ventilation if Needed" parameter, the model then calculates whether or not the individual would experience respiratory paralysis based on the "Probability of Respiratory Paralysis" parameter. Given the outcome of these two parameters, the "Efficacy of Antitoxin Alone (Mechanical Ventilation Unavailable)" parameter modifies probability of death to the percentages described above.

Rationale:

In this parameter, the time of treatment administration is defined as the first time when antitoxin treatment is initiated. Antitoxin is generally given in one dose at a single time point; however, mechanical ventilation and supportive care would be initiated as needed and continued until no longer necessary.

This parameter establishes the efficacy of antitoxin treatment when mechanical ventilation is not available to prevent death from respiratory failure (a major symptom and cause of death related to botulism).[97] Treated individuals who experience respiratory paralysis (as defined in the "Probability of Respiratory Paralysis" section) will die if a ventilator is not available; therefore, our model assumes 100% mortality for patients who suffer respiratory paralysis without mechanical ventilation. Similarly, if mechanical ventilation is not required, our model assumes 0% mortality in individuals who receive antitoxin alone. This assumption may slightly underestimate the number of treated fatalities. Even when antitoxin and mechanical ventilation are available, deaths can still occur from cardiac arrest or other organ failure;[98] however, we did not find enough information to incorporate any further granularity into our analysis; therefore, we assume that all treated patients without respiratory paralysis will survive.

Duration of Symptoms in Treated Non-Survivors

Since antitoxin treatment is not 100% effective, non-survivors may display an altered symptomatic period when treated. The "Duration of Symptoms in Treated Non-Survivors" parameter was established using non-human primate experimental data. The schematic shown below (Figure 17) illustrates the data that were used to establish the length of the symptomatic period in treated non-survivors.
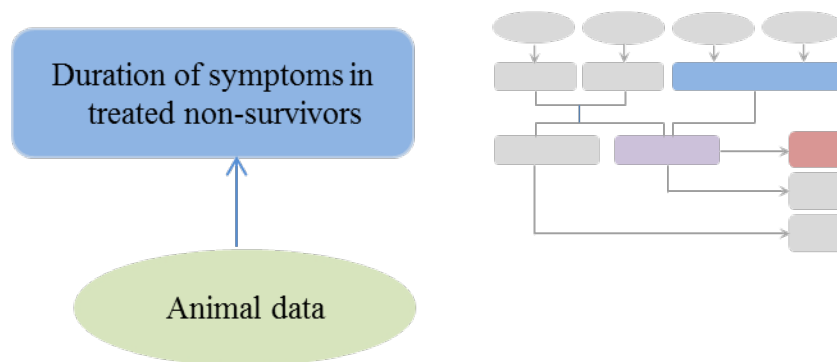

Figure 17. Modeling scheme for the effect of antitoxin on the symptomatic period in non-survivors. The dark blue rectangle indicates the modeling parameter calculations and the green oval indicates data used to establish this parameter. The inset image shows the parts of the larger Figure 1 modeling scheme that are influenced by this parameter.

Value or Function:

Non-survivors treated on the day of death
Symptomatic period = symptomatic period in untreated individual
Non-survivors treated before the day of death
Symptomatic period = normal distribution with an average symptomatic period equal to 400% (SD 265%) of the symptomatic period predicted if the individual were left

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[97] Arnon SS et al. "Botulinum toxin as a biological weapon." *Journal of the American Medical Association.* **285**(1059). 2001.
[98] Tacket CO et al. "Equine Antitoxin Use and Other Factors that Predict Outcome in Type A Foodborne Botulism." *The American Journal of Medicine.***76**. 1984.

untreated. This function is constrained so that the symptomatic period is always greater than or equal to the symptomatic period if the individual were left untreated.

Individuals for Whom this Parameter Applies:

The "Duration of Symptoms in Treated Non-Survivors" parameter modifies the length of the symptomatic period for treated individuals who die. The model first calculates the length of the symptomatic period if the individual were untreated, and then modifies the symptomatic period using the functions described above.

Rationale:

With or without mechanical ventilation, antitoxin treatment is not 100% effective; therefore, for individuals that do not survive, the extent to which treatment will extend the length of the symptomatic period was determined. Data from monkeys that died of botulism despite treatment with antitoxin were used to determine the average increase in the symptomatic period. As shown in Table 5, the increase in the symptomatic period was calculated as a percentage of the time from symptom onset to death of the average control animal (Table A-3, Appendix 2).

The time from symptom onset to death in animals treated with antitoxin was approximately 400% (standard deviation 265%) of the symptomatic period in the average control animal.[99] We assume that the increase in the symptomatic period follows a normal distribution. Additionally, no correlation was found between dose of botulinum neurotoxin and symptomatic period in antitoxin-treated animals; therefore dose is not considered in this parameter (shown in Figure A-1, Appendix 2).

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[99] Although our antitoxin efficacy parameter assumes the availability of supportive care such as feeding tubes and intravenous fluids, only a few animals in our analysis were treated with supportive care - most received antitoxin alone. However, these additional measures generated no significant difference in the symptomatic period; therefore, animals with and without supportive care were included in calculating the parameter. Additionally, those animals treated with antitoxin + mechanical ventilation were excluded because all animals that received this treatment died, which seems unreasonable given the efficacy of antitoxin alone and the high efficacy of antitoxin + mechanical ventilation in humans (see the "Efficacy of Antitoxin with Mechanical Ventilation if Needed" section).

Table 5. Symptomatic Period in Nine Antitoxin-Treated Monkeys				
Source	**Dose (IV LD$_{50}$s)**	**Time from Symptom Onset to Death (Hrs)***		**Time from Symptom Onset to Death as a % of Controls**
		Treated Animals	**Median of Controls**	
Oberst 1965[100]	4.6	9	20.4	44%
	2.5	97.7	20.4	479%
	2.5	98.5	20.4	483%
	2.5	96.9	20.4	475%
	2.5	59.1	20.4	290%
	3.75	188	20.4	922%
	3.75	118	20.4	578%
	4.9	22	20.4	108%
	4	50.1	20.4	246%
AVERAGE				**403%**
STANDARD DEVIATION				**267%**
*In all cases, antitoxin was administered at the same time as, or after the onset of symptoms. *Calculated as the time from the first reported symptom to time of death. **Treatment includes antitoxin with or without supportive care, such as feeding a milk mix intragastrically and providing saline fluids subcutaneously.*				

The average increase in time to death described in Table 5 was based on a variety of treatment start times (ranging from treatment administered immediately after symptom onset to treatment administered after the development of severe symptoms). However, we found no data to specifically support an increase in the symptomatic period for individuals treated on the day of death; thus in our model, the time of death does not change in individuals who die despite treatment administered on the day of death.

Duration of Symptoms in Treated Survivors

Since antitoxin treatment alters the progression of botulism, survivors may display an altered length of the symptomatic period. The "Duration of Symptoms in Treated Survivors" parameter was established using human clinical cases of food-borne botulism. The schematic shown below (Figure 18) illustrates the data that were used to establish the duration of symptoms parameter.

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[100] Oberst FW et al. "Botulinum antitoxin as a therapeutic agent in monkeys with experimental botulism." *US Army Edgewood Arsenal.* CRDLR 3331. 1965.
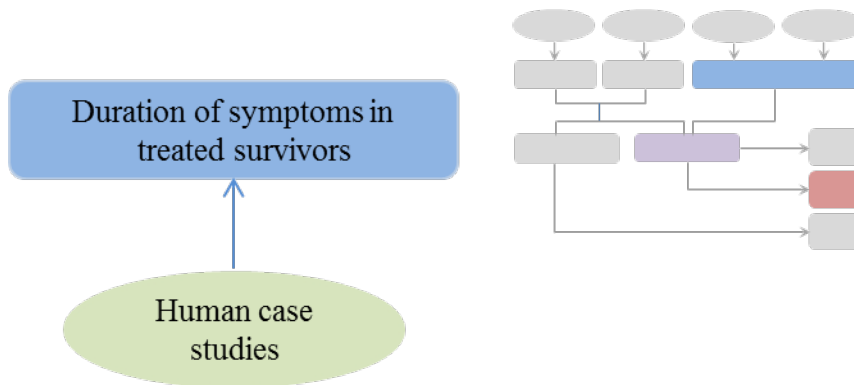
Figure 18. Modeling scheme for the duration of symptoms in treated survivors. The dark blue rectangle indicates the modeling parameter calculations and the green oval indicates data used to establish this parameter. The inset image shows the parts of the larger Figure 1 modeling scheme that are influenced by this parameter.

Value or Function:

> Early-treated survivors:

>> Duration of symptoms (T) is equal to the number of days from symptom onset until no symptoms remain. The duration of symptoms in early treated survivors reported in our model is described by the following functions, where the variable s is a random number greater than or equal to zero and less than or equal to one that determines where in the distribution each individual is placed. (See Calculations and Computational Framework for more information on random numbers):

>> When: $1.562 \times 10^3 * s - 90.00 < 14$, T is equal to 14
>> When: $1.562 \times 10^3 * s - 90.00 > 180$, T is equal to ">180"
>> Otherwise: $T = 1.562 \times 10^3 * s - 90.00$

> Late-treated survivors:

>> Duration of symptoms = Greater than 180 days (">180")

Individuals for Whom this Parameter Applies:

> The "Duration of Symptoms in Treated Survivors" parameter is used to calculate the length of the symptomatic period in symptomatic, treated individuals who survive.

Rationale:

> *Early Treated Survivors*

> To determine the effect of antitoxin on the duration of symptoms in early-treated survivors (those treated within the first day of developing symptoms), we turned to human clinical cases of food-borne botulism. Mann et al. interviewed 27 patients after a large type A food-borne botulism outbreak.[101] Using a written questionnaire, patients recorded their symptoms present at three, six and nine months after treatment (for example, difficulty speaking, double vision, constipation, and dry eyes).

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[101] All but one of the 34 patients in the outbreak received trivalent antitoxin, and 27 patients were contacted and interviewed in the months following treatment. Citation for this statement follows: *Mann JM et al. "Patient recovery from type A botulism: morbidity assessment following a large outbreak." *American Journal of Public Health.* **71**(266-269). 1981.

Our "Duration of Symptoms in Treated Survivors" parameter was established from the Mann et al data under the assumption that an individual is still in the recovery phase of disease (Stage 3) if they have any residual symptoms. Therefore, the duration of symptoms is equal to the number of days after symptom onset when no symptoms remain (as reported by Mann et al). This assumption can be considered conservative since the authors did not specify the type of symptom or the severity of the symptom experienced by patients, and individuals with just one symptom (which could be as minor as dry eyes) were still considered to be symptomatic, and because symptoms were only recorded every three months.

Figure 19 presents the linear regression analysis that was performed to determine the distribution of the duration of symptoms in treated survivors.[102] The equation describing this line in Figure 19 is implemented by generating a random number for s to determine where on the distribution each individual falls.
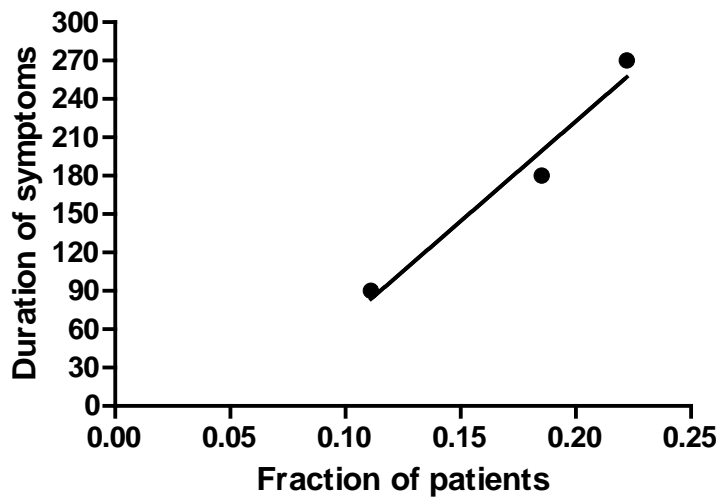

Figure 19. The distribution of the duration of symptoms in treated survivors. The equation of the line is $T = 1.562 \times 10^3 * s - 90.00$, where T is the day after symptom onset, and s is the fraction of early-treated survivors with a duration of symptoms of length T; in our model, the equation is implemented by generating a random number for s to determine where on the distribution the individual falls.

We assumed that if symptoms remain beyond six months, a soldier would not be considered available to his or her unit in a military-relevant timeframe; therefore our model only reports duration of symptoms up until six months after symptom onset. The duration of symptoms in individuals who are calculated to have symptoms lasting longer than six months is reported as greater than 180 days.

In addition, we set a minimum time for the duration of symptoms based on the length of the hospital stay generally required. One study of antitoxin-treated patients found that individuals

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[102] It is important to note, however, that the linear increase in number of patients with no symptoms is likely not valid for longer than the study period (nine months after symptom onset). For example, one other study of food-borne botulism patients one to six years after symptom onset still found remaining symptoms in a significant portion of the patient population. Therefore we do not believe that extrapolating the time of recovery beyond the study period is valid. Citation for this statement follows: *Gottlieb SL et al. "Long-term outcomes of 217 botulism cases in the Republic of Georgia." *Clinical Infectious Diseases.* **45**(174-180). 2007.

under the age of 60 who were treated early with antitoxin had a median hospital stay of 12 days,[103] whereas another source found that the median duration of treatment was seven days (range 5-14 days).[104] Based on these data, we estimated that even in very mild cases of botulism, a minimum of two weeks of recovery are required before no symptoms remain.

Late Treated Survivors

Although it is clear that recovery from botulism is possible in treated individuals, Tacket et al. found that individuals treated more than one day after the onset of symptoms took longer to show sustained improvement and had longer hospital stays as compared to individuals treated early.[105] Since the majority of survivors who were treated early take more than six months to recover and survivors treated late (more than one day after symptom onset) take longer to recover than those treated early, we assumed that patients who survive botulism after being treated late in the disease course will not recover within six months. We therefore assume that the duration of symptoms in all late-treated survivors will be greater than 180 days.

Antitoxin Adverse Effects

The "Antitoxin Adverse Effects" parameter establishes the rate of major and minor adverse effects of antitoxin treatment. Though antitoxin is the only available treatment for botulinum neurotoxin poisoning, it can cause adverse reactions due to host immune response to the foreign equine antibodies. These adverse effects can include: anaphylaxis (an immediate shock-like syndrome caused by a systemic immune reaction), serum sickness (a delayed systemic hypersensitivity reaction caused by large amounts of foreign antigen) and other minor symptoms such as hives.[106] To mitigate the risk of anaphylaxis, patients are screened for hypersensitivity with small doses of antitoxin before receiving a full dose, and those who display an adverse reaction can be desensitized over the course of a few hours by injecting small amounts of the equine antibody, before the full dose is administered.[107] The "Antitoxin Adverse Effects" parameter was established using human clinical studies. The schematic shown below (Figure 20) illustrates the data that were used to establish the antitoxin efficacy modeling parameter.

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[103] Tacket CO et al. "Equine antitoxin use and other factors that predict outcome in type A foodborne botulism."*American Journal of Medicine.***76**(794-798). 1984.

[104] Zhang S et al. "Multilocus outbreak of foodborne botulism linked to contaminated sausage in Hebei province, China." *Clinical Infectious Disease.* **51**(322-325). 2010.

[105] Tacket CO et al. "Equine antitoxin use and other factors that predict outcome in type A foodborne botulism."*American Journal of Medicine.***76**(794-798). 1984.

[106] Janeway CA et al. "Allergy and Hypersensitivity." *Immunobiology.* 5th Edition. Garland Publishing, New York. 2001.

[107] Arnon SS et al. "Botulinum toxin as a biological weapon." *Journal of the American Medical Association.* **285**(1059). 2001.
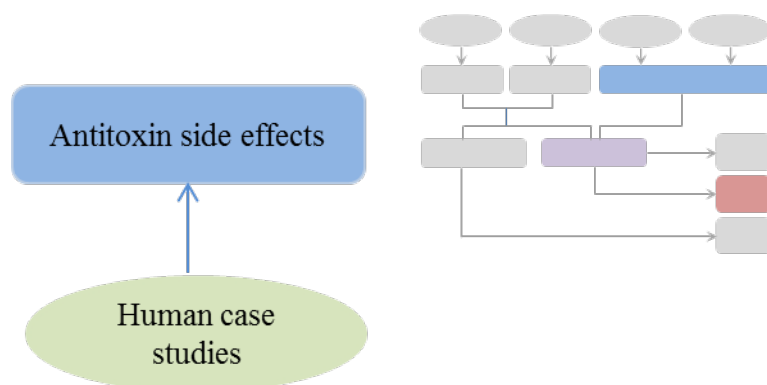
Figure 20. Modeling scheme for antitoxin adverse effects. The dark blue rectangle indicates the modeling parameter calculations and the green oval indicates data used to establish this parameter. The inset image shows the parts of the larger Figure 1 modeling scheme that are influenced by this parameter.

Value or Function:

Table 6. Antitoxin Adverse Effects, Given as Frequency of Treated Individuals	
Side Effect of Antitoxin	**Frequency Seen in Treated Individuals**
Hives	2%
Serum Sickness	2%
Anaphylaxis	1%

Individuals for Whom this Parameter Applies:

The "Antitoxin Adverse Effects" parameter is applied to all individuals who develop symptoms, receive antitoxin treatment and live.

Rationale:

The rates of hives, serum sickness, and anaphylaxis were estimated from human case studies of antitoxins similar to the treatment currently in use (heptavalent botulinum antitoxin, or HBAT). HBAT is comprised of equine Fab/F(ab')$_2$ IgG antibody fragments.[108] The IgG protein is "despeciated" to remove the species-specific Fc region of the antibody, leaving the antigen-specific Fab regions intact. Despeciation reduces the chance that the human immune system will react to the horse-derived antibodies. Although there are no publicly available safety studies on the HBAT antitoxin,[109] clinical studies of the adverse effects of older equine-derived antitoxins that had not undergone despeciation were conducted.

Over 11 years, Black et al. collected physician-reported data on hypersensitivity reactions in 233 patients treated with botulinum antitoxin of equine origin. The study included the trivalent ABE antitoxin, the monovalent E antitoxin, and the bivalent AB antitoxin. The reported reactions included hives, serum sickness, and anaphylaxis.[110] We excluded individuals who were treated

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[108] Fagan RP et al. "Initial recovery and rebound of Type F intestinal colonization botulism after administration of investigational heptavalent botulinum antitoxin." *Clinical Infectious Diseases.* E-publication ahead of print. September 6, 2011.

[109] Fagan R. Personal communication, September 8, 2011.

[110] Black RE and Gunn RA. "Hypersensitivity reactions associated with botulinal antitoxin." *American Journal of Medicine.* **69**(4). 1980.

with more than 40 mL of antitoxin and individuals for whom the dose of antitoxin administered was not recorded. The former were excluded because 40 mL of antitoxin is more than twice the current recommended dose,[111,112] and the latter were excluded because we could not be sure that their dose was less than 40 mL. Of 197 people included in our analysis, five experienced hives, four experienced serum sickness, and five experienced anaphylaxis.

MacDonald et al. also reported on the use of trivalent ABE antitoxin. The antitoxin was administered to 20 people after a type A food-borne botulism outbreak in 1983. Only one individual experienced serum sickness several days after treatment, and no acute hypersensitivity reactions occurred.[113]

The final relevant study of antitoxin adverse effects was conducted by Hibbs et al. after a type E food-borne botulism outbreak in Egypt in 1991.[114] To treat those affected by the outbreak, the US Army supplied an investigational heptavalent F(ab')$_2$ immune globulin of equine origin (dBIG) specific to type A, B, C$_1$, D, E, F, and G toxins. This antitoxin, although different from HBAT, was similarly despeciated to remove the Fc immunoglobulin fragment. Out of 45 patients who received dBIG, none experienced anaphylaxis, one had hives, and one is believed to have developed serum sickness three hours after administration. Eight patients had other minor reactions (local skin reactions, itchiness, and shivering).

In the three studies described above, six patients experienced hives, and six patients experienced serum sickness out of a total of 298 antitoxin-treated individuals. There were no major differences between the rates of hives or serum sickness between the various antitoxins administered in the different studies; we therefore estimate the rate of both hives and serum sickness to be 2%. The rate of anaphylaxis, however, was 2.5% in the individuals who received older equine toxins (antibodies from the Black study and the MacDonald study that had not undergone despeciation), and 0% in the Hibbs study using an antibody despeciated in a similar way to HBAT. Since we would expect the processing of the antibody to significantly decrease the likelihood of an adverse immune reaction, we have estimated the frequency of anaphylaxis to be 1%, which is closer to that of the similarly despeciated antibody.

Although anaphylaxis can lead to death, the prompt administration of epinephrine, diphenhydramine, and intubation can prevent fatalities and thus are part of the emergency protocol when administering antitoxin.[115] Since antitoxin is an injected drug, it will most likely be administered in a setting where anaphylaxis could be quickly and effectively treated, and thus we assume that anaphylaxis would not result in death. Although anaphylaxis could result in an increased rate of death if the proper drugs and equipment are unavailable, the treatment of adverse effects is beyond the scope of this project. For this reason, our model reports the rate of anaphylaxis, but does not include anaphylaxis-related mortality in model outputs.

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[111] Hatheway CH et al. "Antitoxin levels in botulism patients treated with trivalent equine botulism antitoxin to toxin types A, B, and E." *Journal of Infectious Disease.* **150**(407-412). 1984.

[112] State of Alaska. "New recommendations for use of Heptavalent Botulinum Antitoxin (H-BAT)." March 3, 2010. http://146.63.173.6/bulletins/docs/b2010_05.pdf. Accessed on December 20, 2011.

[113] MacDonald KL et al. "Type A Botulism from Sauteed Onions. Clinical and Epidemiological Observations." *Journal of the American Medical Association*. **253**(9). 1985.

[114] Hibbs RG et al. "Experience with the use of an investigational F(ab')2 heptavalent botulism immune globulin of equine origin during an outbreak of Type E botulism in Egypt." *Clinical Infectious Diseases.* **23**(2). 1996.

[115] State of Alaska. "New recommendations for use of Heptavalent Botulinum Antitoxin (H-BAT)." March 3, 2010. http://146.63.173.6/bulletins/docs/b2010_05.pdf. Accessed on December 20, 2011.

Loss of Work

Since there is no recommended post-exposure prophylaxis for botulinum neurotoxin inhalation (as described in the "Introduction and Purpose" and the "Assumptions: Base Case" sections), it is not necessary to consider the amount of work lost due to prophylaxis. Likewise, although antitoxin treatment requires entry into the hospital and therefore would make a soldier unavailable to his or her unit, the model does not consider loss of work due to treatment. This is due to the fact that the amount of time required for administration of treatment (a single injection) and adverse effects of treatment (which are either immediate or occur within a few days of antitoxin injection) are negligible in comparison to the time required for recovery from the effects of the illness.[116] Indeed, survivors of botulinum neurotoxin poisoning often experience a prolonged convalescence, and experience long-term health problems even after recovery (described in detail below) that can lead to loss of work. In the "Loss of Work" parameter we therefore consider whether or not survivors of botulinum neurotoxin poisoning are capable of recovering and returning to work in a time frame relevant to military operations. The "Loss of Work" parameter was established using human clinical studies and data from AMedP-8(C).[117] The schematic shown below (Figure 21) illustrates the data that were used to establish the "Loss of Work" modeling parameter and how this parameter fits into the larger Figure 1 modeling scheme.
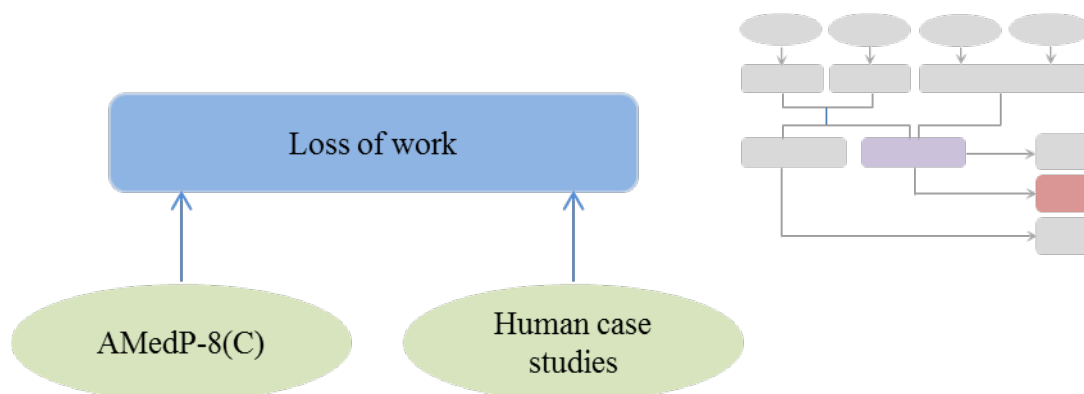

Figure 21. Modeling scheme for loss of work. The dark blue rectangle indicates the modeling parameter calculations and the green ovals indicate data used to establish this parameter. The inset image shows the parts of the larger Figure 1 modeling scheme that are influenced by this parameter.

Value or Function:
> *Untreated survivors:*
> No return to work
>
> *Late-treated survivors (survivors treated more than one day after symptom onset):*
> No return to work
>
> *Early-treated survivors (survivors treated during the first day after symptom onset):*
> Days of work lost equal to the duration of the symptomatic period; if the length of the symptomatic period is greater than six months, then no return to work.

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[116] State of Alaska. "New recommendations for use of Heptavalent Botulinum Antitoxin (H-BAT)." March 3, 2010. http://146.63.173.6/bulletins/docs/b2010_05.pdf. Accessed on December 20, 2011.
[117] NATO. "AMedP-8(C) NATO Planning Guide for the Estimation of CBRN Casualties Ratification Draft 1." February 2010.

Individuals for Whom this Parameter Applies:

The "Loss of Work" parameter is applied to all individuals who become symptomatic and survive.

Rationale:

The "Loss of Work" parameter describes the number of days of work lost by individuals that develop botulism symptoms and survive. AMedP-8(C) indicates that the third stage of disease in untreated survivors, the recovery phase, will last for six months.[118] We assume that a warfighter who is in the recovery phase will be unable to work and that individuals who are incapacitated for six months or more will not return to active duty. Our MCM model therefore assumes that untreated survivors do not return to work. Data in the literature, however, suggest that some individuals who receive treatment will be able to return to work in fewer than six months.

As described in the "Duration of Symptoms in Treated Survivors" section, we used a study of food-borne type A botulism cases to determine the number of days of symptoms suffered by survivors who were treated early after symptom onset.[119] That study described how many days after symptom onset were required until treated individuals no longer had any symptoms. We assumed that when an individual is no longer suffering any symptoms, he or she would be able to return to work. Thus, we assumed that the number of work days lost is equal to the duration of symptoms. Taking into account that warfighters are required to be highly physical, we assumed that an individual with symptoms lasting longer than six months would not be able to return to work; thus survivors who were treated early but suffered from symptoms lasting longer than six months would not return to work.

As described in the "Duration of Symptoms in Treated Non-Survivors" section, we assume that survivors treated late (more than one day after symptom onset) following symptom onset will have symptoms for longer than six months. Given our assumption that six months of symptoms would prevent a warfighter from returning to active duty, we assumed that survivors treated late would not return to work.

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[118] NATO. "AMedP-8(C) NATO Planning Guide for the Estimation of CBRN Casualties Ratification Draft 1." February 2010.
[119] Mann JM et al. "Patient recovery from type A botulism: morbidity assessment following a large outbreak." *American Journal of Public Health.* **71**(266-269). 1981.

Calculations and Computational Framework

The sections below describe the calculations and computational framework of our Excel-based MCM model for botulinum neurotoxin. The influence of user inputs (including dose, vaccination inputs, and treatment inputs) on modeling calculations is described. The framework for the stochastic MCM model is then outlined as a step-by-step process, including a description of the incorporation of random number generators.

How User Inputs Influence Model Calculations

The botulinum neurotoxin model described in this document displays intermediate and final outcomes for each individual based on the underlying parametric values described in the previous section. Intermediate outcomes include whether or not symptoms develop, the timing and duration of those symptoms, and whether respiratory paralysis occurs. Final outcomes include survival or death, work lost due to illness and whether or not treatment adverse effects occur (hives, serum sickness, and anaphylaxis). These intermediate and final outcomes for each individual inputted into the model are displayed on the "Outputs" tab, and are listed in Table 7 below.

Table 7. Modeling Outputs for Each Individual (Outputs Tab)	
Output	**Display Options**
Symptoms	Symptoms/No Symptoms
Time of symptom onset	Days after exposure/NA
Duration of symptoms	Number of days/>180/NA
Respiratory paralysis	Yes/No/NA
Outcome	Recovered/Dead/No Symptoms
Time to death	Days after exposure/NA
Days of work lost	Number of days/No Return to Work/NA
Hives	Yes/No/NA
Serum Sickness	Yes/No/NA
Anaphylaxis	Yes/No/NA
The option "NA" is displayed if the output is not applicable (for example, time of symptom onset in an individual who does not experience symptoms.)	

The "Graphs" tab outputs a summary of the outcomes for all exposed individuals by compiling the data that are reported on the "Outputs" tab. These summarized outcomes include the total number of exposed individuals, the number who develop symptoms, the number who die, the number with respiratory paralysis, the number of survivors who return to work, the number of survivors who do not return to work within six months, the total number of days of work lost by all survivors who do return to work, and the number of survivors who have adverse effects due to MCM. The summary of outcomes for the total exposed population is presented in Table 8 below. The "Graphs" tab also includes a pie chart and several line graphs. The pie chart displays the percentage of individuals who did not experience symptoms, the percentage that recovered after illness, and the percentage that died. The line graphs show the time after exposure when individuals in the population first experience symptoms and when they die.

Table 8. Summary of Outcomes for the Exposed Population (Graphs Tab)
Total number exposed
Number who develop symptoms
Number dead
Number with respiratory paralysis (survivors and non-survivors)
Number of survivors who return to work
Total days of work lost by survivors who return to work
Number of survivors who do not return to work within six months
Number of survivors with adverse side effects due to MCM

The sections below outline how the user inputs selected for each individual contribute to the intermediate and/or final outcomes for that individual.

Exposure Inputs

As described in AMedP-8(C) for botulinum neurotoxin inhalation, the likelihood of symptom onset and likelihood of mortality without treatment are both dose-dependent. Our model allows users to choose a dose of botulinum neurotoxin (in ng) for each modeled individual. Based on the calculations outlined in AMedP-8(C), our model calculates the likelihood of symptom onset and, in individuals who develop symptoms, the likelihood of death without treatment. For example, if an individual inhales 100 ng of botulinum neurotoxin, our model gives that individual a 50% chance of developing symptoms and if the individual develops symptoms, a 2.4% chance of death if left untreated.

Vaccine Inputs

For each modeled individual, users can select whether that individual is vaccinated with four doses of vaccine ("Complete"), vaccinated with only the first three doses of the vaccine ("Incomplete") or not vaccinated at all ("NA"), If "Complete" vaccination is selected, the probability of an individual becoming ill is reduced by 98%. If "Incomplete" vaccination is selected, an additional user input is required to indicate the number of weeks prior to exposure that the last dose of vaccine was administered. After incomplete vaccination, the model reduces the probability of becoming ill based on a function relating the timing of the last dose to vaccine efficacy.

Treatment Inputs

For each modeled individual, users can choose what treatment will be available should the patient develop symptoms. Treatment options include no treatment ("NA"), "antitoxin + ventilation" or "antitoxin alone." When treatment is available, the user must also indicate how soon after symptom onset treatment is first made available.

The "Treatment time" input affects the efficacy of antitoxin. As described in the "Parameters" section of this document, early treatment has a higher efficacy than late treatment. The timing of treatment is combined with each individual's time of symptom onset and time of death to determine the likelihood of survival. The timing of treatment also affects whether individuals experience respiratory paralysis. Our

model assumes that respiratory paralysis occurs in all fatal botulism cases, and our antitoxin efficacy parameters indicate that delaying treatment increases the likelihood of mortality. This scenario makes sense physiologically, because delaying treatment allows for additional binding of toxin to nerve endings, thus increasing the likelihood that an individual will experience respiratory paralysis. Therefore, the number of individuals who experience respiratory paralysis increases with delayed treatment.

The treatment type selected by the user ("antitoxin + ventilation" or "antitoxin alone") also affects the efficacy of treatment. Antitoxin alone can only save individuals who do not experience respiratory paralysis. Treatment with antitoxin + ventilation gives individuals who experience respiratory paralysis a chance of survival, and therefore results in more lives saved. Treatment inputs also affect the length of the symptomatic period in both non-survivors and survivors.

The treatment inputs also affect additional outcomes associated with survivors. The time of treatment administration is defined as the time when antitoxin treatment is first initiated. Antitoxin is generally given in one dose at a single time point; however, mechanical ventilation and/or supportive care would be initiated as needed and continue until no longer necessary. If treatment is administered early, survivors may be able to return to work after recovery. In addition, treated individuals have a chance of experiencing adverse effects of the treatment (anaphylaxis, hives, or serum sickness).

Excel Model Computational Framework

The botulinum neurotoxin MCM model is coded in Microsoft Excel. The model uses the previously described parameters to arrive at outcomes for each individual. The model is built in steps, where each step is represented by a calculation tab in the Excel workbook. For ease of use, the calculation tabs are hidden from the user.[120] The first step calculates the outcome for each individual without MCM. The second step adds the effects of the vaccine inputs. Step three incorporates the effects of the treatment parameters. The outputs for each individual's symptoms, time of symptom onset, duration of symptoms, respiratory paralysis, mortality outcome, and time to death are drawn from this tab. Step four calculates additional outcomes, including the days of work lost, and the presence or absence of adverse effects. Below we provide additional detail on the computational framework on each of these tabs as well as the connections between parameter calculations within the model.

Use of Random Number Generators

Many of the parameters established in the previous section give outcomes in the form of probabilities. Since the botulinum neurotoxin MCM model calculates values for individuals, the model uses a random number generator to determine whether or not a given outcome is realized for a particular individual. For example, our parameter section indicates that 2% of survivors will experience serum sickness. For every individual who is treated with antitoxin, the model generates a random number greater than or equal to zero and less than or equal to one. If the random number is less than or equal to 0.02, that individual develops serum sickness. If the random number is greater than 0.02, that individual does not develop serum sickness. The random number generator thereby allows us to convert the probability of an event into a specific outcome for an individual. Incorporating this element of randomness allows us to model the many possible outcomes that one single individual may experience, and allows for a practical way of varying the outcomes of multiple individuals with identical input scenarios.

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[120] Advanced users can access the calculation tabs by right clicking on any tab, selecting "Unhide" and choosing the tab that they wish to view.

Additionally, the model's use of random numbers is designed to keep the results of each calculation step consistent for a single individual; therefore, each individual is assigned a single random number for each outcome that is reliant on a random number. For example each individual has a single random number associated with "lethality." If that random number is 0.6 and the chance of untreated death is 89% (following exposure to 1000 ng of toxin), then individual will die. With treatment (antibiotics + ventilation) the chance of death falls to 59%; since the random number associated with lethality is static for each individual, with treatment this individual will survive.

Step 1: No MCM

Using the exposure data input by the user, the model calculates each individual's outcome if no MCM are administered. This tab recreates the outcomes already established by AMedP-8(C) for the untreated disease.[121] In the absence of MCM, the likelihood of symptoms and the likelihood of death are dependent on the dose indicated by the user. Whether or not each individual experiences symptoms is determined by pairing the individual's dose-dependent probability of developing symptoms with a random number generator. If the model determines that an individual develops symptoms, a second random number is used to convert the probability of respiratory paralysis and death into a final mortality outcome (survival or death). Given the final mortality outcome, the model calculates the duration of symptoms, and if applicable, the time of death. Both the duration of symptoms and time to death in untreated individuals are described by AMedP-8(C).

Step 2: Vaccine

If the user indicates that no vaccine is administered (by selecting "NA"), no change is made to the modeling outcomes in this step. If the user selects "Complete" or "Incomplete" vaccination, the model adjusts the probability of developing symptoms that was calculated on the "No MCM" tab in Step 1. If "Complete" vaccination is selected, the probability of developing symptoms is reduced by 98%. If "Incomplete" vaccination is selected, the probability of developing symptoms is also reduced; however the exact value by which this probability is reduced depends on the number of weeks before exposure that the last vaccine dose is administered (as described in the parameters section). A random number is once again used to convert the probability of symptoms into an outcome (symptoms or no symptoms). Other outcomes (death, respiratory paralysis, loss of work, duration of symptoms, and time to death, if applicable) are recalculated using the same method applied in Step 1. Although it is possible that the severity or duration of illness is altered in vaccinated individuals who develop symptoms, no evidence to support this hypothesis was found in the literature; thus the model does not adjust the disease course based on vaccination status.

Step 3: Treatment

The treatment tab is the final step in determining disease-related outcomes for each individual. None of the outcomes are adjusted for individuals who receive no treatment; furthermore, the symptom/no symptom outcome is not adjusted during this step. The likelihood of death, however, is adjusted for individuals who receive timely treatment (if treatment is administered after the time of death, treatment inputs are ignored). Individuals treated with antitoxin have a decreased chance of developing respiratory paralysis and thus have an increased chance of survival. Those treated with antitoxin + ventilation have an even greater chance of survival since ventilation will allow some individuals who develop respiratory paralysis to survive. As before, the probability of death is converted into an actual outcome (recovery or death) using a random number generator.

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[121] NATO. "AMedP-8(C) NATO Planning Guide for the Estimation of CBRN Casualties Ratification Draft 1." February 2010.

As described in the "Duration of Symptoms in Treated Non-Survivors" section, if antitoxin is administered on any symptomatic day besides the day of death, the duration of symptoms in non-survivors is increased by 400% (SD 265%). The time to death is also adjusted according to the duration of symptoms. The symptomatic period (and thus the time to death) does not change in individuals who die despite antitoxin treatment administered on the day of death.

The length of the symptomatic period in treated survivors is calculated based on the "Duration of Symptoms in Treated Survivors" parameter. For each individual that is treated early (within one day of developing symptoms), the individual is placed at random within the distribution described by this parameter using a random number generator for the variable *s*. The parameter equation is then used to calculate the duration of symptoms, *T*. For each individual that is treated late (greater than or equal to one day after the onset of symptoms), the duration of symptoms is reported as greater than six months.

Step 4: Work Lost and Adverse Effects

If an individual's outcome is death, Step 4 is not calculated. If an individual's outcome is survival, the model calculates outcomes for work lost and treatment adverse effects. As described in the "Parameters" section, the number of days of work lost is equal to the duration of symptoms. For individuals who have symptoms for longer than six months (including untreated survivors), the model reports "No Return to Work."

The final outcomes for the adverse effects of treatment are determined by combining the probability of each outcome (anaphylaxis, serum sickness, and hives) with a random number generator. Since adverse effects occur soon after the administration of antitoxin, our model does not include adverse effects in the number of days of work lost. We assume that any adverse effects would occur during the minimum duration of symptoms (14 days) which is equal to the minimum number of days of work lost.

Sample Results

This model of MCM efficacy against botulinum neurotoxin allows the user to input any combination of dose, pre-exposure vaccination, and treatment for each individual modeled. Below, we provide results from a few sample runs to demonstrate the range of outcomes the model will produce with varying inputs. The outcomes of each scenario include pie graphs divided into three categories: the "Not Sick" portion indicates the individuals that were exposed but did not develop symptoms; the "Recovered" portion indicates the individuals that developed symptoms and survived; the "Dead" population indicates the individuals that developed symptoms and died. As mentioned previously, the model calculates each individual's outcome independently and stochastically, such that each time the model is run, the output may differ even if the input parameters do not change.

Dose Variation (No MCM)

In this sample model run, the outcomes of three scenarios are compared in which the dose of toxin is varied and individuals receive no MCM. In scenarios one, two, and three, each individual is exposed to 10, 100 and 1,000 ng of toxin, respectively (Table 9). Since these outputs do not include MCM, the outputs are based solely on the parameters described by AMedP-8(C).

Input

Table 9. Input for Exposure to Varying Doses of Botulinum Neurotoxin - Modeling Example			
	Scenario 1	**Scenario 2**	**Scenario 3**
Number of exposed individuals	500 individuals	500 individuals	500 individuals
Inhaled dose per person	10 ng of toxin	100 ng of toxin	1,000 ng of toxin
Vaccine	No	No	No
Treatment	No	No	No

Output

a. Outcome 1 — Not Sick 100%, Recovered 0%, Dead 0%
b. Outcome 2 — Not Sick 50%, Recovered 50%, Dead 0%
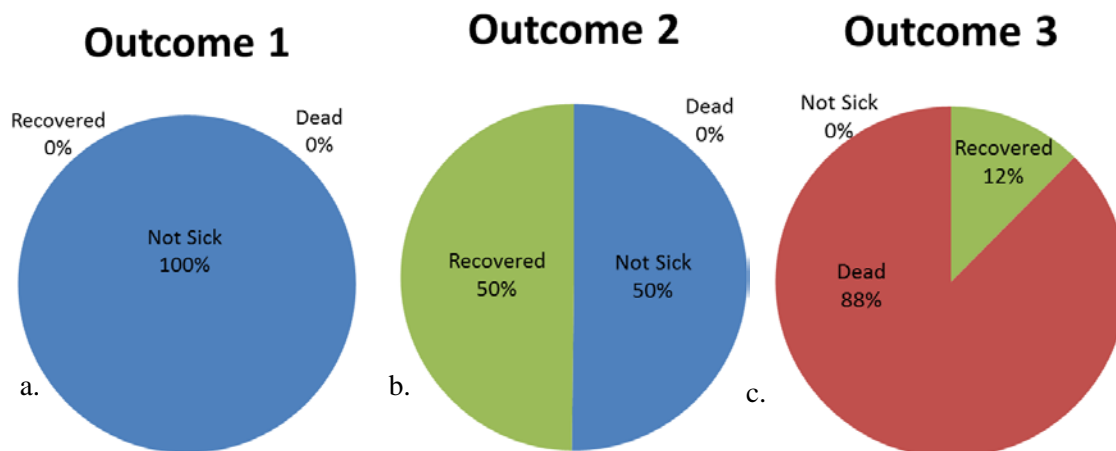c. Outcome 3 — Not Sick 0%, Recovered 12%, Dead 88%

Figure 22. Outcomes for individuals exposed to (a) 10 (b) 100 or (c) 1,000 ng of botulinum neurotoxin receiving no MCM.

Analysis

These results show that individuals exposed to small doses of toxin may not develop symptoms at all (Figure 22). As the dose of inhaled toxin increases, so does the likelihood that each individual will develop symptoms. According to AMedP-8(C), the likelihood that an untreated symptomatic individual will die is also dose-dependent. In scenario one, none of the exposed individuals develop symptoms. As the dose of agent increases, the likelihood of symptoms and the likelihood of death both also increase.

Vaccination

Vaccination with the recently discontinued PBT vaccine can prevent the onset of symptoms and we assume that any vaccine developed in the future will be at least as efficacious as PBT. However, the level of protection falls off with time in individuals who are not fully vaccinated (have not receive four doses of vaccine). In this section, modeling outputs from three scenarios with different vaccination schemes are compared. In the first scenario, unvaccinated individuals are exposed to 1,000 ng of botulinum neurotoxin. This scenario serves as a baseline for comparison with scenario two, where fully vaccinated individuals were exposed to 1,000 ng of botulinum neurotoxin (the same dose as in scenario one). In the third scenario, individuals are partially vaccinated, and received their third dose of the vaccine 26 weeks before exposure (Table 10).

Input

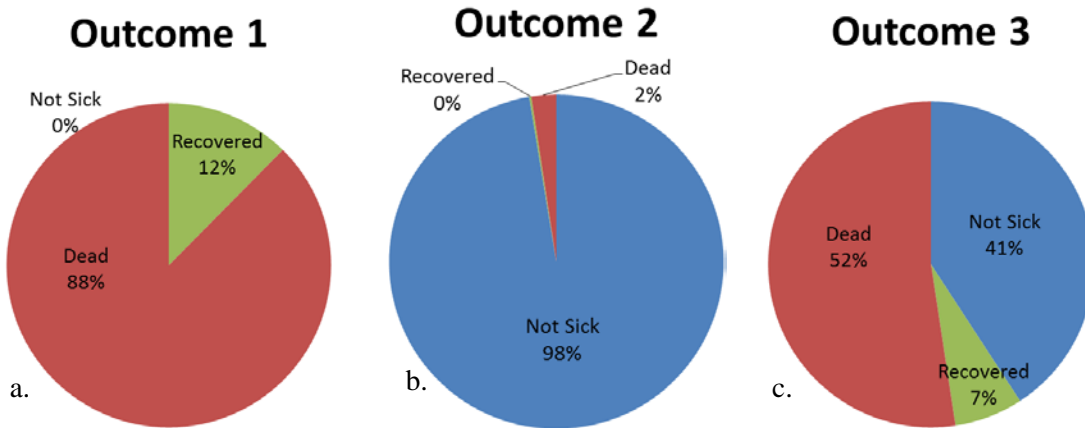| Table 10. Input for Pre-exposure Vaccination - Modeling Example			
	Scenario 1	**Scenario 2**	**Scenario 3**
Number of exposed individuals	500 individuals	500 individuals	500 individuals
Inhaled dose per person	1,000 ng of toxin	1,000 ng of toxin	1,000 ng of toxin
Vaccine	No	Complete	Incomplete; last dose 26 weeks before exposure
Treatment	No	No	No

Output

Figure 23. Outcomes for (a) unvaccinated individuals exposed to 1,000 ng of botulinum neurotoxin, (b) fully vaccinated individuals exposed to 1,000 ng of botulinum neurotoxin and (c) partially vaccinated individuals who received their third dose of vaccine 26 weeks before exposure to 1,000 ng of botulinum neurotoxin.

Analysis

The model output demonstrates that the vaccine can prevent the onset of symptoms, but is less effective when individuals have not received the full series of shots (Figure 23). The outputs do not show any difference in a symptomatic individual's chance of dying (the ratio of recovered to dead is similar in all three scenarios). While we hypothesize that in a real-life scenario, vaccinated individuals who develop symptoms will have an increased chance of survival, we were unable to find any data to support this hypothesis and it is thus not reflected in our model.

Treatment with Antitoxin Alone

In this example, we compare three scenarios in which individuals are exposed to botulinum neurotoxin and receive no MCM until after they develop symptoms. In scenario one, individuals are exposed to 10,000 ng of botulinum neurotoxin and receive no MCM. In scenarios two and three, each individual receives antitoxin starting one, or three days after symptom onset (Table 11). The high dose of toxin used in this scenario allows us to compare the effect of treatment on mortality rate in a population that would most likely die if left untreated.

Input

| Table 11. Input for Treatment with Antitoxin - Modeling Example			
	Scenario 1	**Scenario 2**	**Scenario 3**
Number of exposed individuals	500 individuals	500 individuals	500 individuals
Inhaled dose per person	10,000 ng of toxin	10,000 ng of toxin	10,000 ng of toxin
Vaccines	No	No	No
Treatment	No treatment	Antitoxin started 1 day after symptom onset	Antitoxin started 3 days after symptom onset

Output

a.   b.   c.
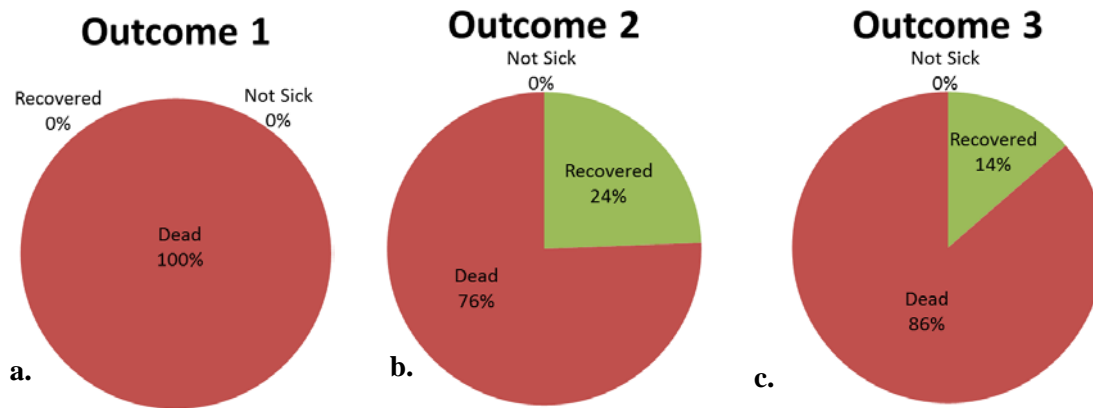
Figure 24. **Outcomes for individuals exposed to 10,000 ng of botulinum neurotoxin that (a) received no antitoxin, (b) received antitoxin 1 or (c) 3 days after the onset of symptoms.**

Table 12. Work Lost Due to Illness			
	Outcome 1	**Outcome 2**	**Outcome 3**
Number of survivors who do not return to work*	0	102	68
Number of survivors who return to work*	0	20	0
Days of work lost in survivors who return to work*	0	1686	0
*Within six months of symptom onset			

Analysis

As shown in scenario one above, individuals who inhale 10,000 ng of antitoxin and receive no MCM will most likely die (Figure 24). The administration of antitoxin one day after the onset of symptoms significantly improves the probability of recovery, although the majority of individuals will still perish. Delaying antitoxin administration until the third day of symptoms decreases an individual's chances of survival even further. This is partially due to the parameter which accounts for the data showing that

antitoxin is most effective when given in the first 24 hours of symptom and also due to the fact that by day three, some individuals will have already died.

The work lost outputs of the model provide information on the availability of a warfighter to return to duty after convalescence (Table 12). Since our model assumes that the possibility of return to work is only available to individuals treated early (within one day of symptom onset), only scenario two results in survivors who return to work. The model also reports the total number of days lost collectively by the survivors who are able to return to work. In untreated and late-treated individuals, our model assumes that no one returns to work within six months; therefore, no survivors in scenarios one and three return to work.

Treatment with Antitoxin and Ventilation

When administered before the onset of respiratory paralysis, antitoxin treatment can often prevent further paralysis, and therefore prevent death. However, when antitoxin does not prevent respiratory paralysis, mechanical ventilation can be used to keep individuals alive, while the toxin works its way out of the system, thus restoring respiratory function. Below, we present three treatment scenarios: in scenario one, individuals are not treated; in scenarios two and three, individuals are treated with antitoxin and ventilation (if needed) beginning one or three days after symptom onset (Table 13). This example is the same as the example presented above for treatment with antitoxin alone, except in this example treated individuals are given ventilation if they experience respiratory failure.

Input

| Table 13. Input for Treatment with Antitoxin and Ventilation - Modeling Example			
	Scenario 1	**Scenario 2**	**Scenario 3**
Number of exposed individuals	500 individuals	500 individuals	500 individuals
Inhaled dose per person	10,000 ng of toxin	10,000 ng of toxin	10,000 ng of toxin
Vaccines	No	No	No
Treatment	No treatment	Antitoxin + ventilation (if needed) started less than 1 day after symptom onset	Antitoxin + ventilation (if needed) started 3 days after symptom onset

Output
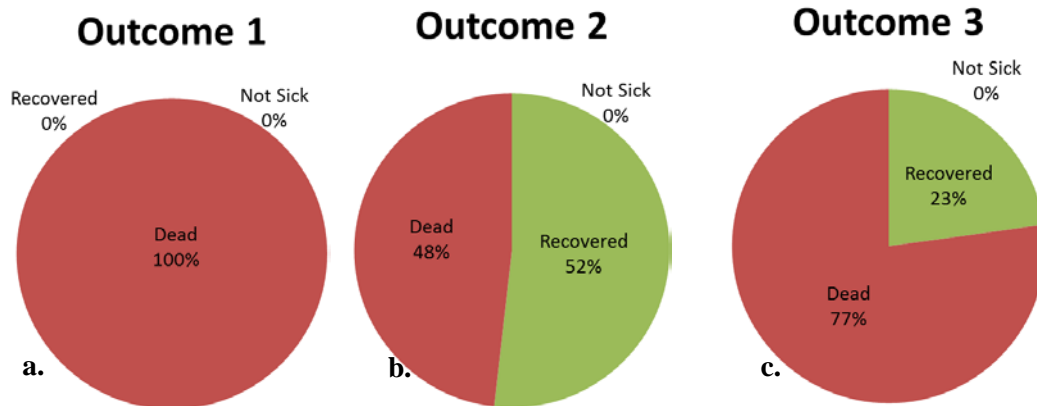
Figure 25. Outcomes for individuals exposed to 10,000 ng of botulinum neurotoxin that (a) received no antitoxin, (b) received antitoxin (and ventilation if needed) 1 or (c) 3 days after the onset of symptoms.

Table 14. Work Lost Due to Illness			
	Outcome 1	**Outcome 2**	**Outcome 3**
Number of survivors who do not return to work*	0	213	114
Number who return to work*	0	46	0
Days of work lost in survivors who return to work*	0	3420	0
Within six months of symptom onset			

Analysis

The pie charts above show that administering botulinum antitoxin and, if needed, ventilation one day after symptom onset can prevent approximately half of deaths resulting from exposure to 10,000 ng of botulinum neurotoxin. While individuals who do not receive these treatments until three days after the onset of symptoms are more likely to survive than untreated individuals, only about half as many individuals will recover as when treated the day after symptom onset. By comparing these charts with those from the previous examples, the benefit of mechanical ventilation becomes clear. Individuals treated with antitoxin and mechanical ventilation are about twice as likely to recover than those treated with antitoxin alone.

The work lost outputs of the model provide information on the availability of warfighters to return to duty after convalescence. As described for antitoxin alone, our model assumes that the possibility of return to work is only available in individuals treated early (within one day of symptom onset), and therefore only scenario two includes survivors who return to work. For survivors in scenarios one and three (untreated and late-treated), our model assumes that they do not return to work within six months. In comparison to the number survivors treated with antitoxin alone (previous example scenarios two and three), the number of survivors who are treated with antitoxin and ventilation (this example scenarios two and three) is greater; therefore, the number of survivors in each survivor category (do not return to work, number who return to work, and days of work lost in those who return to work) is correspondingly greater (Table 13 versus Table 14).

Run to Run Variation

Our model of the efficacy of various countermeasures against botulinum neurotoxin is probabilistic and stochastic. The outcome for each individual is established using evidence-based parameters, probability distributions, and an element of randomness, which determines where in the probability distribution an individual falls. Therefore, two individuals with identical characteristics may experience different outcomes in the model. Specifically, given a description of agent exposure, vaccination, or treatment, the model calculates the likely outcome in terms of morbidity, mortality, adverse effects, and loss of productivity. The evidence-based parameters underlying the model determine the probability of each outcome; the model then uses a random number to convert this probability into an outcome for each individual.

Because the model incorporates an element of randomness, there is run-to-run variability in the outcomes. The extent of this variation is closely tied to the parameters that define each input scenario. Scenarios with parameters for which the probability of an event is 0% and scenarios with parameters for which the probability is 100% will have no variation in the outcome, since any random number drawn by the model will give the same outcome. As with a coin toss, each random number draw is independent of the previous draw (see the section on "Use of Random Number Generators"). For example, it is possible (if unlikely), that ten people in a row will remain symptom-free following inhalational exposure to botulinum neurotoxin, even if each has a 50% chance of developing symptoms. Figure 26 demonstrates that the closer the predicted chance of symptoms is to 50%, the greater the standard deviation between runs of 100 identical individuals.
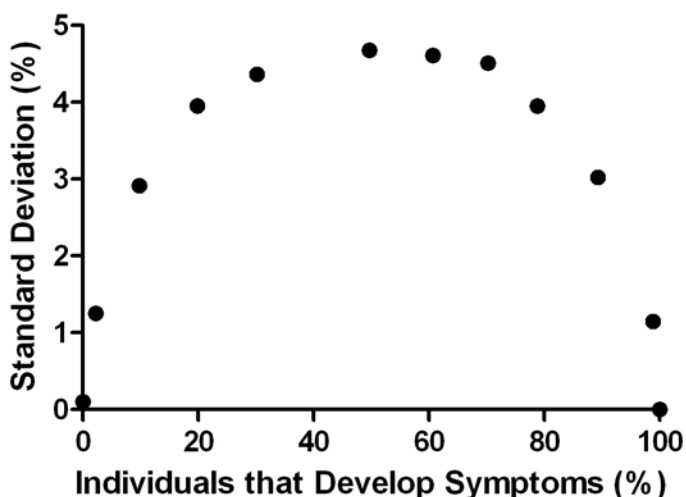

Figure 26. The relationship between the probability of developing symptoms and standard deviation of outcomes, within a population of 100 individuals.

Users can reduce the standard deviation of the outcome by running the model for larger populations of individuals. For example, at its most variable (a probability of developing symptoms of 50%, a dose of 100 ng[122]), the standard deviation of the number of individuals who develop symptoms in a model run with 100 individuals is 10%, but drops to 1% with 10,000 individuals. Table 15 below demonstrates how, in a sample model run, the standard deviation decreases as the number of identical people increases.

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[122] NATO. "AMedP-8(C) NATO Planning Guide for the Estimation of CBRN Casualties Ratification Draft 1." February 2010.

Table 15. Effect of Population Size on Standard Deviation			
Number of individuals	**100**	**1,000**	**10,000**
Mean number of symptomatic individuals	49.789	497.89	4978.9
SD (people)	4.959342	16.39099	56.69695
SD (%)	10%	3%	1%
Scenario - Inhaled Dose: 100 ng; No MCM.			

Modeling runs that contain few identical individuals are likely to have significant run-to-run variation. Users who wish to produce outcomes closer to the average should take the mean of a large number of individuals with identical inputs, either by running many individuals at once or taking the average of several identical runs.

Conclusions

The Microsoft Excel botulinum neurotoxin model described in this work estimates the morbidity, mortality, course of disease, and work lost in individuals following exposure to aerosolized botulinum neurotoxin. The model was built using untreated disease course parameters developed by AMedP-8(C)[123], and evidence-based countermeasure efficacy parameters developed by Gryphon Scientific. This model is intended to be used as a tool to estimate outcomes and determine needs in a military population exposed to aerosolized botulinum neurotoxin. Using this model, military planners can better understand the vulnerabilities of warfighters, the benefit of countermeasures, and logistical tradeoffs on the battlefield.

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[123] NATO. "AMedP-8(C) NATO Planning Guide for the Estimation of CBRN Casualties Ratification Draft 1." February 2010.

Appendix 1. Untreated Disease Course

This appendix contains additional information on the untreated disease course. Described below are the data used to establish a standard deviation for the "Duration of the Symptomatic Period" parameter in untreated survivors.

Standard Deviation of Untreated Survivors

The onset and duration of botulism symptoms in surviving monkeys, as reported by Herrero et al., were analyzed in order to establish a standard deviation for the duration of symptomatic Stage 1 and Stage 2 in untreated survivors.[124] Stage 1 was defined as the duration of time between the onset of ptosis and the onset of muscular weakness, and Stage 2 was defined as the duration of time between the onset of muscular weakness and the initiation of recovery, i.e. the day on which the animal first stopped displaying any previously observed symptom. Table A-1 below shows the calculated durations of Stage 1 and Stage 2 of each surviving monkey. Based these data, the standard deviation of Stage 1 is 112% of the mean length of Stage 1, and the standard deviation of Stage 2 is 61% of the mean length of Stage 2.

Table A- 1. Duration of Symptomatic Stages in Rhesus Monkeys Administered Botulinum Neurotoxin Intravenously						
		Days Post-Exposure				
Monkey No.	**Dose (MU/kg)***	**Ptosis Onset**	**Muscular Weakness Onset**	**Initiation of Recovery****	**Calculated Stage 1**	**Calculated Stage 2**
3	44	2.5	3	5.5	0.5	2.5
4	44	2.5	3	9	0.5	6
5	44	3	4	6.5	1	2.5
6	44	2.5	3	4	0.5	1
27	46	1	2	8	1	6
34	52	1.5	3.5	11	2	7.5
55	38	4	6.5	12	2.5	5.5
57	38	4	10.5	12	6.5	1.5
58***	38	4	NA	10.5	NA	NA
Mean					**1.81**	**4.06**
Standard Deviation					**2.03**	**2.46**
Standard Deviation (% of Mean)					**112%**	**61%**
Estimated in 12 hour increments from Herrero et al 1967, Figure 1. ** Mouse units (one mouse intraperitoneal LD50 in mg) per kilogram.* *** Initiation of recovery was defined as the first day that the animal recovered from one of its reported symptoms.* **** It was not possible to calculate the duration of the symptomatic stages for animal #58 because only one symptom (ptosis) was observed.*						

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[124] Herrero BA et al. "Experimental Botulism in Monkeys – A Clinical Pathological Study." *Experimental and Molecular Pathology.* **6**(1). 1967.

Appendix 2. Treatment

This appendix contains additional information on our antitoxin treatment parameters. The sections below include details of the heptavalent botulism antitoxin (HBAT) composition, the probability of respiratory paralysis in food-borne type A botulism, and additional information on the timing of death after treatment.

Toxin Neutralization by HBAT

Our model assumes that one dose of antitoxin is sufficient to neutralize any reasonable inhaled dose of botulinum neurotoxin that can be administered by aerosol exposure. The heptavalent HBAT antitoxin contains 7,500 international units (IU) of serotype type A, 5,500 IU of type B, 5,000 IU of serotype C, 1,000 IU of serotype D, 8,500 IE of serotype type E, 5,000 IU of serotype F, and 1,000 IU of serotype G.[125] Typically, one-tenth of an IU of antitoxin will neutralize at least 1,000 mouse LD_{50}s of type A toxin; thus the heptavalent antitoxin is expected to neutralize 75,000,000 mouse LD_{50}s of type A botulinum neurotoxin.[126] Since the LD_{50} in mice is approximately 1 ng/kg and the average mouse weighs 20g,[127] one dose of antitoxin is expected to neutralize 1.5 mg of type A toxin. Since it is unlikely that a human will be exposed to more than 1.5 mg of aerosolized toxin, our model does not consider a defeat dose.

Probability of Respiratory Paralysis

As stated in the main text, our model assumes that some, but not all, individuals who survive with treatment would experience respiratory paralysis, and would therefore require ventilator assistance. Data from clinical reports of human food-borne type A botulism cases shown in Table A-2 below were used to determine the proportion of treated individuals that require mechanical ventilation, and thus must have experienced respiratory paralysis. In these food-borne outbreaks, 208 individuals were treated with antitoxin and about half (101) of those individuals received mechanical ventilation.[128] Based on these data, our model estimates that 50% of treated individuals that survive will experience respiratory paralysis.[129]

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[125] Fagan RP et al. "Initial recovery and rebound of Type F intestinal colonization botulism after administration of investigational heptavalent botulinum antitoxin." *Clinical Infectious Diseases*. E-publication ahead of print. September 6, 2011.

[126] Mayers CN et al. "Antitoxin therapy for botulinum intoxication." *Reviews in Medical Microbiology*. **12**(1). 2001.

[127] Zhang JC, Sun L and Nie QH. "Botulism, where are we now?" *Clinical Toxicology*. 48. 2010.

[128] One large report of Type A botulism was excluded because ventilator use was reported, but the number of patients that received antitoxin was not. Woodruff BA et al. "Clinical and laboratory comparison of botulism from toxin types A, B, and E in the United States, 1975-1988." *Journal of Infectious Diseases*. **166**(6). 1992.

[129] The data used to develop this parameter may have included some fatalities, because in some cases it was not clear whether or not the cited number of fatalities overlapped with the number of antitoxin-treated patients. Additionally, some studies did not explicitly state whether or not all individuals who received ventilation had also received antitoxin. However, since the number of fatalities was relatively low (14 total reported in all studies included in the model) and since it is standard procedure to administer antitoxin with mechanical ventilation for patients with botulism who experience respiratory paralysis, we assume that these unknowns have a small impact on the rate of ventilator use.

Table A- 2. Ventilator Use in Cases of Food-borne Type A Botulism		
Source	**Received Antitoxin**	**Received Ventilation***
Hatheway 1984[130]	4	1
Kalluri 2003[131]	9	6
King 2008[132]	2	2
MacDonald 1985[133]**	19	11
Mann 1981[134]**	31	8
Tacket 1984[135]**	77	73
Zhang 2010[136]	66	0
TOTAL	**208**	**101**

When not explicitly stated we assumed that individuals who receive ventilation also received antitoxin, since antitoxin is the standard treatment for individuals who have a severe enough form of botulism to require ventilation.[137]

**Individuals that received antitoxin and died were excluded from this analysis, since this parameter is applied only to the population that would survive if given all necessary treatment (antitoxin, mechanical ventilation, and supportive care.)*

Tacket 1984: Table III in this paper appears to contain a typo – the total number of late treated patients is most likely 61 (not 16) with 50 treated survivors. The data cited here includes both early (within one day of symptom onset) and late (more than one day after symptom onset) treated survivors.

[130] Hatheway CH et al. "Antitoxin levels in botulism patients treated with trivalent equine botulism antitoxin to toxin types A, B, and E." *Journal of Infectious Diseases.* **150**(3). 1984.

[131] Kalluri P et al. "An outbreak of foodborne botulism associated with food sold at a salvage store in Texas." *Clinical Infectious Diseases.* **37**(11). 2003

[132] King LA. "Two severe cases of botulism associated with industrially produced chicken enchiladas, France, August 2008." *Eurosurveillance.* **13**(37). 2008.

[133] MacDonald KL et al. "Type A Botulism from Sauteed Onions. Clinical and Epidemiological Observations." *JAMA.* **253**(9). 1985.

[134] Mann JM et al. "Patient Recovery from Type A Botulism: Morbidity Assessment following a Large Outbreak." *American Journal of Public Health.* **71**(3). 1981.

[135] Tacket CO et al. "Equine Antitoxin Use and Other Factors that Predict Outcome in Type A Foodborne Botulism." *The American Journal of Medicine.***76**. 1984.

[136] Zhang S et al. "Multilocus Outbreak of Foodborne Botulism Linked to Contaminated Sausage in Hebei Province, China." *Clinical Infectious Diseases*. **51**(3). 2010.

[137] Center for Disease Control. "Botulism, General Information: How can botulism be treated?" November 11, 2010. Accessed on June 7, 2012. http://www.cdc.gov/nczved/divisions/dfbmd/diseases/botulism/#treat

Symptomatic Period in Untreated Control Animals

In our "Duration of Symptoms in Treated Non-Survivors" parameter in the main text of this document, animal data from a study by Oberst et al[138] were used to show that the average time from symptom onset to death in animals treated unsuccessfully with antitoxin was longer than in untreated control animals. Table A-3 below gives the data used to develop the median time to death in control animals from the same study.

Table A-3. Symptomatic Period in Untreated Control Animals				
Dose	**Exposure to Symptom Onset (hrs)**	**Exposure to Death (hrs)**	**Symptomatic Period**	**Notes**
5	NR	43	-	Excluded because time of symptom onset was not given
5	28	64	36	
5	28.6	49	20.4	
5	34.4	73	38.6	
4.6	29	38	9	
3.75	24	32	8	
3.75	20.3	38	17.7	
3.75	34	49	15	
2.5	Excluded	40	-	Excluded because symptom onset time was given as <38.4 hrs
2.5	NR	135	-	Excluded because time of symptom onset was not given
2.5	39.3	49	9.7	
2.5	38	130	92	
2.5	41.5	67	25.5	Times calculated as the middle of the reported range
2.5	38.2	67	28.8	
MEDIAN			**20.4**	
NR = Not reported.				

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[138] Oberst FW et al. "Botulinum antitoxin as a therapeutic agent in monkeys with experimental botulism." *US Army Edgewood Arsenal.* CRDLR 3331. 1965.

Antitoxin Effect on Symptomatic Period and Dose

In our "Duration of Symptoms in Treated Non-Survivors" parameter described in the main text of this document, animal data were used to show that the average symptomatic period in animals treated unsuccessfully with antitoxin was longer than in untreated control animals. The effect of botulinum neurotoxin dose on the length of the symptomatic period was also examined using the same study listed in the main text.[139] As shown in Figure A-1, we found no correlation between dose and symptomatic period in antitoxin-treated animals. Therefore our model does not considered dose in our "Duration of Symptoms in Treated Non-Survivors" parameter.
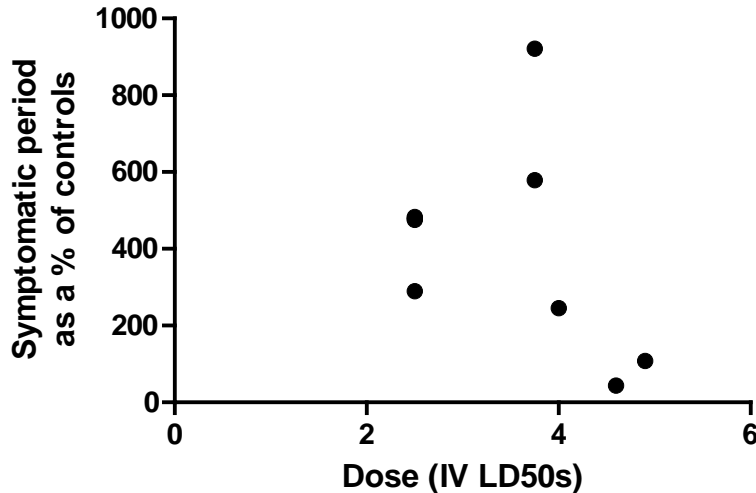

Figure A-1. The symptomatic period of treated animals (given as a percentage of the symptomatic period in control untreated animals) was unrelated to the dose of toxin administered.

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[139] Oberst FW et al. "Botulinum antitoxin as a therapeutic agent in monkeys with experimental botulism." *US Army Edgewood Arsenal.* CRDLR 3331. 1965.