THE EPIDEMIOLOGY AND CLINICAL ASPECTS OF FOODBORNE BOTULISM IN TEXAS, 1992 - 2001

THESIS

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By

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DEDICATION

I dedicate this thesis to my loving wife, Pamela and our beautiful daughters Jessica, Patricia, Anna and Carolie. They have given their full support, with forgiving patience for the time we have lost in my pursuit of an education. I further dedicate this thesis to Irene, the rock of our family, who has supported us in every way. Her loving kindness will be sorely missed. And my thanks to Tom who is always there when we need him.

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Table of Contents

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

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Foodborne botulism, caused by the organism *Clostridium botulinum* is a public health emergency requiring rapid diagnosis and response. Outbreaks of botulism occur sporadically, and there may be no cases reported in Texas for several years at a time. As the most potent organic toxin known, *C. botulinum* toxin has the potential to be used as a biological weapon. Methods used to treat botulism include administration of antitoxin, cathartics, gastric lavage and high enemas. An affected person may spend months on a

ventilator while recovering from foodborne botulism. This study looks at the effect diarrhea may have on the symptoms and outcomes of botulism. Diarrhea may occur in about one half of the cases, and may be caused by other organisms found in the implicated food. Study subjects were identified from two foodborne outbreaks of botulism. With age and sex as covariates, the relationship between diarrhea (independent variable) and various symptoms and outcomes (dependent variables), was examined with the use of odds ratios, logistic regression and other multivariate techniques. Study results indicated an association between diarrhea and fatigue (odds ratio (OR) = 6.29, 95% confidence interval (CI) 1.19 - 33.35). Frequency of neurologic symptoms and interventions were lower in those with diarrhea. Patients with diarrhea were less likely to be hospitalized (OR = 0.76, 95% CI = 0.16 - 3.58), but this association was not significant at the 0.05 level. Diarrhea was not significantly associated with any other symptoms or outcomes.

CHAPTER I

INTRODUCTION

Background

Clostridium botulinum is a relatively large, gram-positive, rod-shaped bacterium that grows endospores and has a strictly fermentative mode of metabolism. It will not grow under aerobic conditions and vegetative cells are killed by exposure to oxygen, but the spores are able to survive long periods of exposure to air. It is common in the anaerobic habitats of nature where organic compounds are present, including soils, aquatic sediments, and the intestinal tracts of animals (Todar, 2002). *C. botulinum* is actually a group of organisms that are alike only in that they are clostridia and produce antigenically distinct neurotoxins which are the most toxic biologic substances known to affect humans. There are seven types of *C. botulinum*, which are distinguished by the antigenic characteristics of the neurotoxins that they produce (Centers for Disease Control, 1998; Solomon & Lilly, 1998; Maksymowych, Reinhard, Malizio, Goodnough, Johnson, & Simpson, 1999; Todar, 2002). Types A, B, E, and in rare cases, F are toxic to humans, while types C and D cause disease in birds and nonhuman mammals. Type G has not yet been confirmed as a cause of illness in humans or animals (Centers for

Disease Control, 1998). Four distinct forms of botulism can occur: foodborne, wound, infant, and that resulting from intestinal colonization of a person older than 1 year of age.

The ability of *C. botulinum* to cause food poisoning in humans is directly related to the production of heat-resistant spores that survive preservation methods that kill nonsporulating organisms. The heat resistance of spores varies from type to type and even from strain to strain within each type. The spores of many strains require temperatures above boiling to ensure destruction. The thermal resistance of spores also increases with higher pH and lower salt content of the medium in which the spores are suspended (Centers for Disease Control, 1998; Solomon & Lilly, 1998). Most control methods focus on the inhibition of growth and toxin production. The main limiting factors for growth of *C. botulinum* in foods are: (1) temperature, (2) pH, (3) water activity, (4) redox potential, (5) food preservatives, and (6) competing microorganisms (Centers for Disease Control, 1998). All of these factors are interrelated and so changing one factor influences the effect of other factors (Centers for Disease Control, 1998; Solomon & Lilly 1998).

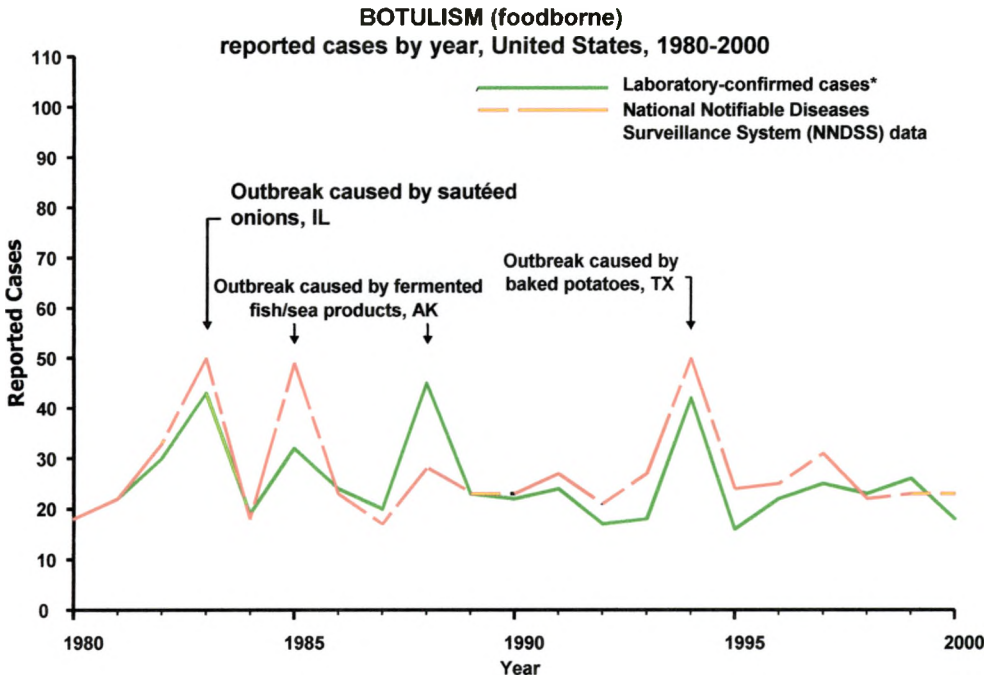
The interaction of factors may have a positive or negative effect on the inhibition of *C. botulinum*. In general, proteolytic strains grow optimally at 40°C; the lower limit is 10°C, upper limit is 45-50°C. Nonproteolytic strains, including type E can continue to grow even at 3.3°C. The minimum pH range for growth of proteolytic strains is 4.6-4.8; the limit is pH 5.0 for nonproteolytic strains (Centers for Disease Control, 1998; Solomon & Lilly 1998). However, some food proteins, such as soy and beef, may have a protective effect on *C. botulinum* at or below pH 4.6. In addition, certain food preparations may contain low-acid "pockets" in which the pH may be high enough to

support the production of toxin. Low water activity (aw) inhibits the growth of *C. botulinum*. A minimum aw of ~0.94 is needed to support growth and toxin production. Water activity can be limited by dehydration, but is in general controlled by the addition of NaCl. The minimum aw of 0.94 corresponds to an approximate 10% NaCl solution. High redox potential (Eh) is usually due to the presence of oxygen. The optimum Eh for growth of *C. botulinum* is low (~-350 mV) but toxin production has been observed at Eh of +250 mV. Because of this range, *C. botulinum* growth and toxin production can occur even in products considered to have a high oxygen level. In addition, vacuum packaging used to lower Eh to preserve food increases anaerobic conditions and so may support the production of toxin. A number of food preservatives (nitrite, sorbic acid, parabens, phenolic antioxidants, polyphosphates and ascorbates) inhibit the growth of *C. botulinum* and limit toxin production. Lactic acid bacteria such as *Lactobacillus*, *Pediococcus*, and *Lactococcus* have been shown to produce acid and so inhibit *C. botulinum* (Centers for Disease Control, 1998).

Epidemiology

In the U.S. Type A is the most significant cause of botulism, involved in 37.6% of foodborne botulism outbreaks since 1950 (Centers for Disease Control, 1998). Outbreaks stand out from the background rate because of the rarity of the disease (see Figure 1).

Figure 1.

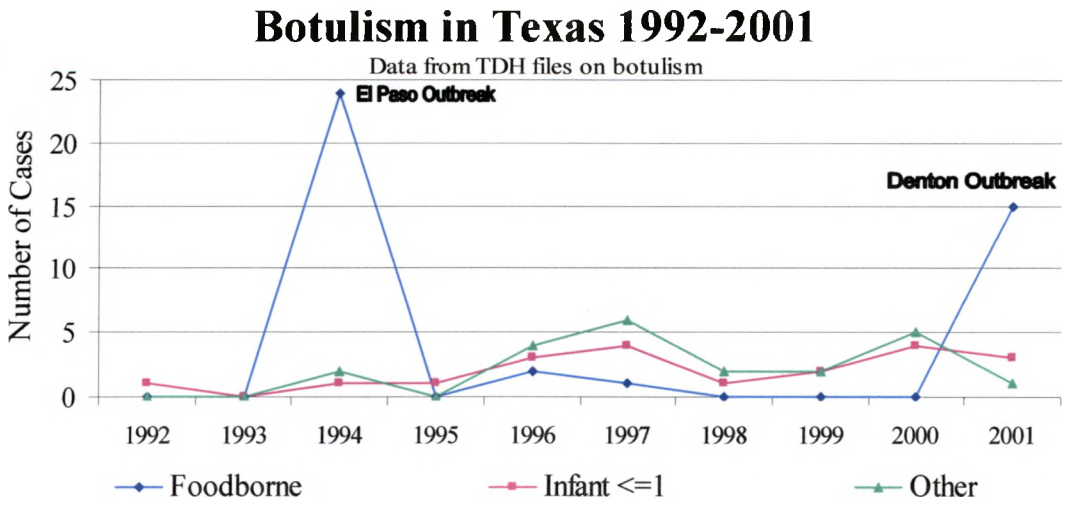


*Data from annual survey of State Epidemiologists and Directors of State Public Health Laboratories.
MMWR Summary of Notifiable Diseases, United States, (2000)

Although this type of food poisoning is rare, the mortality rate is high; the 962 recorded botulism outbreaks in the United States from 1899 to 1990 involved 2320 cases and 1036 deaths (Solomon & Lilly, 1998). For the period 1899-1949, the case fatality rate was high, at approximately 60%, but since about 1950, mortality has gradually decreased to 15.5% for the period 1950-1996. This decline in the case fatality rate is due primarily to improvements in supportive and respiratory intensive care and presumably to the prompt administration of antitoxin (Centers for Disease Control, 1998). The first patient in an outbreak has a 25% chance of death, whereas subsequent cases, (who are diagnosed and treated more quickly), carry only a 4% risk of such an outcome (Todar, 2002).

In Texas, there were 42 cases of foodborne botulism in the ten years prior to 2002. Males accounted for 23 of the foodborne cases and females accounted for 19. The age distribution for foodborne botulism was as follows: 7 people from 1 to 19 years, 15 people from 20 to 40 years, 16 people from 41 to 60 years and 4 people over 60 years of age. The racial distribution was 26 whites, 15 blacks and 1 of unknown ethnicity. There were 20 cases of infant botulism, also known as floppy baby syndrome, which is caused by the colonization of the intestinal tract of a defenseless baby resulting in a subsequent intoxication. This included 10 male and 10 female babies of whom there were 15 whites and 5 babies of unknown ethnicity. The category labeled "other" comprises wound botulism, intestinal colonization of a person older than 1 year of age, and those whose cause is unknown. In the last ten years, there were 22 cases classified as other. This included 10 males and 12 females. Of these, 20 people were between the ages to 1 to 19 years and 2 were between 20 and 40 years of age. There were 15 whites, 1 black and 6 of unknown ethnicity (TDH files on botulism, 2001).

Figure 2



Foodborne botulism is rare but it may kill rapidly, and contaminated commercial products may expose many persons. There is also the potential for the toxin to be used as an agent of bioterrorism. Botulinum toxin poses a major bioweapon threat because of its extreme potency and lethality; its ease of production, transport, and misuse; and the need for prolonged intensive care among affected persons. A single gram of crystalline toxin, evenly dispersed and inhaled, would kill more than 1 million people, although technical factors would make such dissemination difficult. Timely recognition of a botulism outbreak begins with an astute clinician who quickly notifies public health officials. Diagnosis of a single botulism case is a public health emergency that requires immediate intervention including prompt provision of botulinum antitoxin and, often, mechanical ventilation (Arnon S. S. & Schechter R. 2001).

Clinical Aspects

The clinical syndrome of botulism is dominated by the neurologic signs and symptoms resulting from a toxin-induced blockade of the voluntary motor and autonomic cholinergic junctions and is quite similar for each cause and toxin type (Centers for Disease Control, 1998). The structure and mechanism of action of each of the seven neurotoxins are similar. Each toxigenic *Clostridium* produces a polypeptide of 150kDa which is activated by proteases following bacterial lysis. The active toxin consists of a heavy chain (H, 100 kDa) and a light chain (L, 50 kDa). The heavy chain consists of an amino-terminal 50 kDa domain (H_N) and a carboxy-terminal 50 kDa domain (H_C) (Centers for Disease Control, 1998; Maksymowych & Simpson 1998; Todar, 2002). In foodborne botulism, the toxin is absorbed by the upper part of the gastrointestinal tract in

the duodenum and jejunum, and passes into the blood stream by which it reaches the peripheral neuromuscular synapses (Todar, 2002). Neuronal cell intoxication occurs through four steps: (1) binding of H_C to polysialoganglioside receptors on the neuronal membrane, (2) internalization of active toxin into endosomal-like compartments, (3) membrane translocation facilitated by H_N and (4) enzymatic cleavage of target proteins by the L chain to prevent release of the neurotransmitter acetylcholine from synaptic terminals of the motor neurons in muscle (Centers for Disease Control, 1998; Maksymowych & Simpson, 1998).

Clinical symptoms of botulism usually begin 18-36 hours after toxin ingestion. The primary symptom is progressive descending weakness or flaccid paralysis starting in the face, with dizziness and dryness of the mouth (Centers for Disease Control, 1998; Todar, 2002). The ingestion of other bacteria or their toxins in the improperly preserved food or changes in bowel motility are likely to account for the abdominal pain, nausea, vomiting, and diarrhea that often precede or accompany the neurologic symptoms of foodborne botulism (Centers for Disease Control, 1998). Neurologic signs and symptoms soon develop such as blurred vision, inability to swallow, difficulty in speech, descending weakness of skeletal muscles and respiratory paralysis leading to ventilatory failure and death (Centers for Disease Control, 1998; Todar, 2002). Recovery follows the regeneration of new neuromuscular connections. The differential diagnosis includes myasthenia gravis, stroke, Guillain-Barré syndrome, hypokalemia, bacterial and chemical food poisoning, tick paralysis, and chemical intoxication (Centers for Disease Control, 1998).

Purpose of Study

The mainstays of treatment for foodborne botulism include; administration of botulinum antitoxin in an attempt to prevent neurologic progression of a rapidly progressive illness; careful monitoring of respiratory vital capacity and aggressive respiratory care for those with ventilatory insufficiency; and meticulous and intensive care for the duration of the often prolonged paralytic illness (Centers for Disease Control, 1998). Those who are known to have eaten the incriminated food should be purged with cathartics and given gastric lavage and high enemas (Centers for Disease Control, 2002).

This treatment regimen is performed to eliminate toxin from the GI tract and raises the question: Does diarrhea mitigate the outcomes of cases by decreasing the time toxin remains in the gastrointestinal tract and therefore reduce the amount of toxin absorbed? To answer that question, the research hypothesis of this thesis and the null hypothesis are the following:

- Hypothesis: Acute diarrhea in persons exposed to incriminated food will improve the outcomes of botulism cases.
- Null hypothesis: Acute diarrhea in persons exposed to incriminated food has no effect on the outcomes of botulism cases.

It seems logical that decreasing the time the food containing toxin is in the gastrointestinal tract, will lower the amount of toxin that is absorbed. It would also seem logical to think that acute onset of diarrhea, brought on by the consumption of the offending food, would decrease the time the toxin is present in the gastrointestinal tract.

To test this hypothesis, data collected by the Texas Department of Health for two retrospective cohort studies of botulism were combined and reanalyzed.

CHAPTER II

METHODS

In April 1994, the largest outbreak of botulism in the United States since 1978 occurred in El Paso, Texas. Thirty persons were affected after eating food from a Greek restaurant. The attack rate among persons who ate a potato-based dip was 86%. The attack rate among persons who ate an eggplant-based dip was 67%. Botulism toxin type A was detected from patients and in both dips. Toxin formation resulted from holding aluminum foil-wrapped baked potatoes at room temperature, apparently for several days, before they were used in the dips (Texas Department of Health, 1995). Another outbreak occurred in August of 2001 in the Dallas area. Fourteen persons were affected after eating at a church supper. The attack rate among persons who ate chili was 58%. Botulism toxin type A was detected from patients and chili. Toxin formation occurred as a result of packaged frozen chili being allowed to thaw and then being refrozen if not sold during the week (Kalluri, 2001).

Research Design

Both studies were pursued using standard epidemiological methods. These methods include determining the extent of the outbreak; identifying the contaminated food; establishing the cause of contamination; and proposing control measures. EpiInfo was used for both studies to create questionnaires, collect and store data, and analyze the data. The questionnaires used in the two outbreaks differed primarily in the enumeration of the foods in question. The demographics and symptoms in both questionnaires as well as the type of response (yes/no) were similar. This thesis study is a retrospective cohort analysis of the association between the occurrence of diarrhea and other symptoms and outcomes among the cases during the two outbreaks.

Case Definition

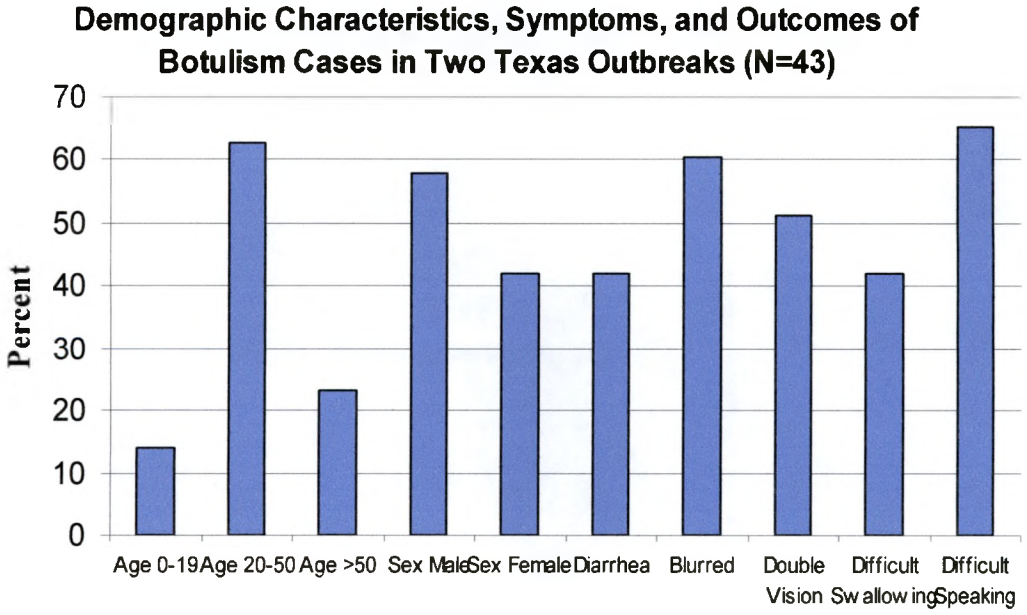
The case definitions for foodborne botulism were:

- *Probable*: a clinically compatible case (cranial neuropathy, gastrointestinal illness) with an epidemiologic link.
- *Confirmed*: a clinically compatible case that is laboratory confirmed or that occurs among persons who ate the same food as persons who have laboratory-confirmed botulism (Centers for Disease Control, 1998).

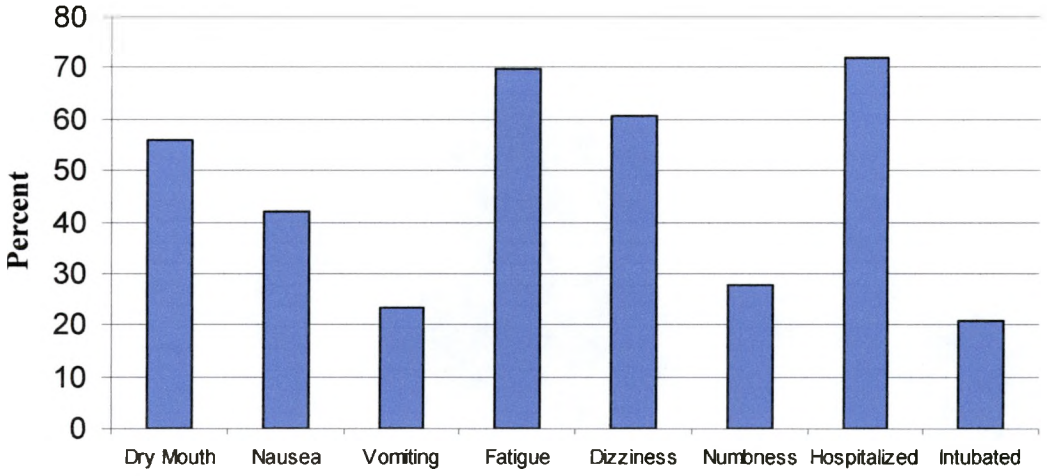
Study Population

The two study cohorts consisted of 44 cases that included 24 confirmed and 20 probable. Only cases were used in the analysis because symptoms and outcomes were used as the dependent variables. One case from another town was excluded. A total of 13 symptom variables including diarrhea were studied. Of the cases, 58% were male and 42% were female. The symptoms were distributed among the cases as follows: 42% had diarrhea, 60.5% had blurred vision, 51.2% had double vision, 41.9% had difficulty swallowing, 65.1% had difficulty speaking, 55.8% had dry-mouth, 41.9% had nausea, 23.3% had vomiting, 69.8% had fatigue, 60.5% had dizziness, 27.9% had numbness, 69.8% were hospitalized and 20.9% were intubated.

Figure 3



Demographic Characteristics, Symptoms, and Outcomes of Botulism Cases in Two Texas Outbreaks (N=43)



Analysis

Using SPSS 11.0, the data were analyzed for odds ratios including 95% confidence limits and p-values to measure the association between diarrhea and the observed symptoms and outcomes. Logistic regression was performed using diarrhea, sex, and age as independent variables. In a first analysis, hospitalization was the dependent variable and in a second analysis intubation was used as the dependent variable. These analyses were used to determine if there was a significant relationship between diarrhea and possible outcomes requiring intervention. The effects of age and sex were also evaluated. A new set of seven interval variables were created to represent logical combinations of the original categorical variables in order to have interval data to work with. The frequencies of positive responses of the combinations of original variables were summed to create the new variables. The construction of the new variables of summed positive responses consisted of the following:

- Vision = blurred vision + double vision
- Oral = difficult swallowing + difficult speaking + dry mouth
- Systemic = fatigue + dizziness + numbness
- Intervention = hospitalized + intubated
- Neurological = blurred vision + double vision + difficult swallowing + difficult speaking + dry mouth + numbness
- Gastrointestinal = nausea + vomiting
- Total = sum of all symptoms present

Using SPSS 11.0, crosstabs of these new variables with the presence of diarrhea (yes/no) were performed. Standardization of the new variable scores was achieved by dividing the new variable score by the number of old variables of which the score was composed. A bar chart was created in order to visualize relationships of the new variables. Multivariate linear regression and analysis of covariance was performed using the General Linear Model function of SPSS with diarrhea as a fixed factor and age and sex as covariates. The set of standardized new variables were used as dependent variables to determine main effects and interactions and to examine the overall relationship between diarrhea and various combinations of symptoms or outcomes.

CHAPTER III

RESULTS

Association of Symptoms and Outcomes of Botulism with Diarrhea

The only symptom significantly associated with diarrhea was fatigue, which had an odds ratio of 6.29, and a confidence interval between 1.19 and 33.35 ($p < 0.05$). This association indicated that botulism cases with diarrhea had a greater chance of complaining of fatigue. Diarrhea may have reduced the rate of hospitalization, as indicated by an odds ratio of 0.63 but the 95% confidence interval included 1.0 and this association did not achieve statistical significance ($p > 0.05$). None of the other symptoms or outcomes was significantly associated with diarrhea. Odds ratios ranged between 1.45 and 2.75 with all 95% confidence intervals containing 1.0.

Table 1

Association of Symptoms/Outcomes with Diarrhea, Two Texas Botulism Outbreaks							
Symptom/Outcome	Cases with diarrhea (18)		Cases without diarrhea (25)		Odds ratio	95% Confidence Interval	
	N	%	N	%		Lower	Upper
Blurred vision	12	66.2	14	56.0	1.6	0.4	5.5
Double vision	11	61.1	11	44.0	2.0	0.6	6.9
Difficult swallowing	9	50.0	9	36.0	1.8	0.5	6.1
Difficult speaking	14	77.8	14	56.0	2.8	0.7	10.7
Dry mouth	11	61.1	13	52.0	1.5	0.4	5.0
Nausea	9	50.0	9	36.0	1.8	0.5	6.1
Vomiting	6	33.3	4	16.0	2.6	0.6	11.2
Fatigue	16	88.9	14	56.0	6.3	1.2	33.3
Dizziness	13	72.2	13	52.0	2.4	0.7	8.8
Numbness	7	38.9	5	20.0	2.5	0.7	10.0
Hospitalization	12	66.2	19	76.0	0.6	0.2	2.4
Intubation	5	27.8	4	16.0	2.0	0.5	8.9

Logistic Regression Analysis

The logistic regression analysis of factors related to hospitalization indicated that age was a significant negative predictor. Younger people were more likely to be hospitalized. Sex and diarrhea were not significant predictors of hospitalization however. The model had a correct prediction rate of 72.1%. Age, sex and diarrhea were not significantly associated with intubation.

Table 2
Logistic Regression Results of Age, Sex, and Diarrhea as Predictors for Hospitalization, Botulism cases

								95 0% C I for EXP(B)	
		B	S E	Wald	df	Sig	Exp(B)	Lower	Upper
Step 1 [a]	DIARRHEA	- 274	790	120	1	729	760	162	3 577
	AGE	- 052	024	4 745	1	029	949	906	995
	SEX	- 846	845	1 002	1	317	429	082	2 248
	Constant	3 461	1 485	5 434	1	020	31 856		

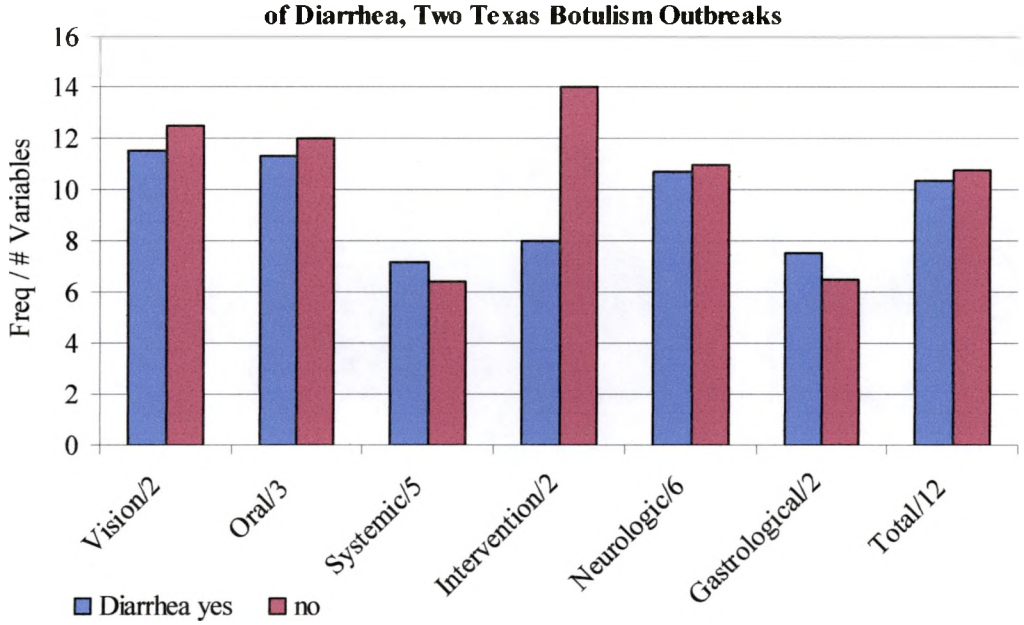
[a] Variable(s) entered on step 1 DIARRHEA, AGE_____, SEX_____

Frequencies of Symptoms and Outcomes in Relation to Diarrhea

The results of the crosstabs of diarrhea with the standardized new variables are shown in Figure 4 and display the relative number of those with and those without diarrhea in each of the standardized new variables. It is apparent that those with diarrhea had fewer symptoms in all categories but two, Systemic/5 and Gastrointestinal/2. These are the two categories not only related to neurological symptoms. These findings make sense when you consider that the variables Systemic/5 and Gastrointestinal/2 are both related to diarrhea. Systemic/5 contains the frequency of positive responses to fatigue, which has a significant association with diarrhea in the odds ratio analysis. Gastrointestinal/2 contains the frequency of positive responses to nausea and vomiting, two symptoms which often occur when diarrhea is caused by infection. It is clear in the two cohorts that neurological symptoms are less frequent in those who have diarrhea. The greatest difference between groups is the category Intervention/2, which contains the outcomes of hospitalization and intubation. In these two cohorts, those with diarrhea were less likely to require intervention.

Figure 4

Standardized Frequencies of Symptoms and Interventions by the Presence of Diarrhea, Two Texas Botulism Outbreaks



Multivariate ANCOVA (General Linear Model)

In the multivariate Analysis of Covariance of the standardized variables, diarrhea and sex did not have a significant effect on symptoms and outcomes in the overall model. Age was very close to being a significant effect with a p-value of 0.058.

Table 3
Multivariate Tests of Effects of Age, Sex and Diarrhea on Symptoms and Outcomes

Effect		Value	F	Hypothesis df	Error df	Sig
Intercept	Pillai's Trace	241	2 223	5 000	35 000	074
	Wilks' Lambda	759	2 223	5 000	35 000	074
	Hotelling's Trace	318	2 223	5 000	35 000	074
	Roy's Largest Root	318	2 223	5 000	35 000	074
AGE_____	Pillai's Trace	254	2 389	5 000	35 000	058
	Wilks' Lambda	746	2 389	5 000	35 000	058
	Hotelling's Trace	341	2 389	5 000	35 000	058
	Roy's Largest Root	341	2 389	5 000	35 000	058
SEX_____	Pillai's Trace	096	741	5 000	35 000	598
	Wilks' Lambda	904	741	5 000	35 000	598
	Hotelling's Trace	106	741	5 000	35 000	598
	Roy's Largest Root	106	741	5 000	35 000	598
DIARRHEA	Pillai's Trace	126	1 012	5 000	35 000	425
	Wilks' Lambda	874	1 012	5 000	35 000	425
	Hotelling's Trace	145	1 012	5 000	35 000	425
	Roy's Largest Root	145	1 012	5 000	35 000	425

In the tests of between-subjects effects, diarrhea and sex do not have a significant effect for any of the standardized variables. Age does show a significant effect on Intervention/2 with a p-value of 0.03. The parameter for age on Intervention/2 indicates that older persons were less likely to require intervention. R-squared for age is only 0.147, a low value indicating that very little is explained by that effect.

Table 4 Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig
Corrected Model	vision/2	247[a]	3	8 242E-02	409	747
	oral/3	710[b]	3	237	1 742	174
	intervention/2	721[c]	3	240	2 238	099
	neurological/6	433[d]	3	144	1 375	265
	total/12	469[e]	3	156	2 094	117
Intercept	vision/2	869	1	869	4 312	044
	oral/3	4 563E-02	1	4 563E-02	336	565
	intervention/2	856	1	856	7 972	007
	neurological/6	199	1	199	1 891	177
	total/12	335	1	335	4 492	040
AGE_____	vision/2	3 089E-02	1	3 089E-02	153	697
	oral/3	155	1	155	1 141	292
	intervention/2	545	1	545	5 079	030
	neurological/6	1 237E-02	1	1 237E-02	118	733
	total/12	2 893E-02	1	2 893E-02	388	537
SEX_____	vision/2	5 863E-03	1	5 863E-03	029	865
	oral/3	412	1	412	3 032	090
	intervention/2	5 503E-02	1	5 503E-02	513	478
	neurological/6	189	1	189	1 802	187
	total/12	176	1	176	2 361	132
DIARRHEA	vision/2	223	1	223	1 106	299
	oral/3	126	1	126	928	341
	intervention/2	1 080E-02	1	1 080E-02	101	753
	neurological/6	190	1	190	1 810	186
	total/12	223	1	223	2 986	092
Error	vision/2	7 857	39	201		
	oral/3	5 295	39	136		
	intervention/2	4 186	39	107		
	neurological/6	4 094	39	105		
	total/12	2 909	39	7 460E-02		
Total	vision/2	21 500	43			
	oral/3	18 667	43			
	intervention/2	13 750	43			
	neurological/6	15 444	43			
	total/12	13 715	43			
Corrected Total	vision/2	8 105	42			
	oral/3	6 005	42			
	intervention/2	4 907	42			
	neurological/6	4 527	42			
	total/12	3 378	42			

[a] R Squared = 031 (Adjusted R Squared = - 044)

[b] R Squared = 118 (Adjusted R Squared = 050)

[c] R Squared = 147 (Adjusted R Squared = 081)

[d] R Squared = 096 (Adjusted R Squared = 026)

[e] R Squared = 139 (Adjusted R Squared = 072)

CHAPTER V

DISCUSSION

Study Limitations and Strengths

Unfortunately the results of this investigation are not strong enough to draw any conclusions. The size of the combined cohort was not large enough to achieve the statistical power required to determine whether the presence of diarrhea reduced symptoms or resulted in less serious outcomes in botulism cases. PEPI samples program option 1, when given the relative parameters from the analysis in Table 1, suggested a cohort of 672 persons to achieve a power of 90% and a significance of 0.05, to look at hospitalization. To look at intubation with the same level of confidence would require 365 persons. The type of data collected and the reliability could have been better. For instance, diarrhea should have been quantitated by the number of loose stools per day as it is for *E. coli* investigations. This more precise definition would have allowed better determination of the severity of diarrhea as it relates to symptoms and outcomes. The data on constipation were not complete for one of the cohorts; therefore it was necessary to eliminate it from the analysis. As a counterpoint to diarrhea, constipation is important because it can indicate the arrival of neurological symptoms. Often a patient will have diarrhea and then become constipated as the neurotoxin takes effect. In comparing the

data with the original questionnaires, there were some inconsistencies. Two of the intubated cases did not indicate that they were hospitalized. That is impossible of course; therefore the computer data were corrected to be consistent with the hard copy. One person was unsure if they had diarrhea so a negative response was entered. This was a conservative judgment since if their diarrhea was significant they would have likely reported so. Neither of these changes had a significant effect on the results. Data for both cohort studies were collected by the same investigators with the use of very similar procedures; which are strengths of this study.

Possible Explanation

After reviewing the literature on the subject of botulinum toxin, the results are not surprising. The speed at which an organism succumbs to a foodborne toxin is related to the strength of the toxin and the rate of uptake by the gastrointestinal system. The strength of botulinum toxin is second to none, requiring as little as 100 billionths of a gram (10^{-11}g) for a fatal dose, making the toxin one of the most lethal poisons known. Several studies have recently been published concerning the mechanisms involved in the uptake and transport of botulinum toxin to the nerve endings where the damage is done. To test the hypothesis that pure neurotoxin can be absorbed and to gauge the role of auxiliary proteins in this process, a series of experiments were done in mice by Maksymowych (Maksymowych, Reinhard, Malizio, Goodnough, Johnson, & Simpson, 1999). Three neurotoxin preparations were examined as follows: (1) pure neurotoxin, (2) neurotoxin in a complex that contained hemagglutinins, and (3) neurotoxin in a complex that did not contain hemagglutinins. These preparations were injected directly into the

stomachs or intestines of animals with or without ligation of the pylorus. The results of these studies help clarify the efficacy of neurotoxin absorption, both in the presence and in the absence of auxiliary proteins. Comparison of relative toxicities demonstrated that at adequate doses, complex with hemagglutinins, complex without hemagglutinins, and pure neurotoxin can be absorbed from the stomach. The potency of neurotoxin in complex was greater than that of pure neurotoxin, but the magnitude of this difference diminished as the dosage of neurotoxin increased. Qualitatively similar results were obtained when complex with hemagglutinins, complex without hemagglutinins, and pure neurotoxin were placed directly into the intestine. This work establishes that pure botulinum neurotoxin serotype A is toxic when administered orally. This finding indicates that pure neurotoxin does not require hemagglutinins or other auxiliary proteins for absorption from the gastrointestinal system into the general circulation.

Maksymowych conducted an earlier study on the binding and transcytosis of botulinum neurotoxin by polarized human colon carcinoma cells to determine the mechanism and efficacy of uptake of botulinum toxin (Maksymowych & Simpson 1998). Using serotype A as an example, the rate of transcytosis by T-84 cells was determined in both apical to basolateral (11.34 fmol/h/cm2) as well as basolateral to apical (8.98 fmol/h/cm2) directions, and by Caco-2 cells in the apical to basolateral (8.42 fmol/h/cm2) direction. Serotype A retained intact di-chain structure during transit through T-84 or Caco-2 cells, and when released on the basolateral side was toxic in vivo to mice and in vitro on mouse phrenic nerve hemidiaphragm preparations. This study shows that botulinum toxin is actively transported across intestinal epithelium at a rapid rate.

Conclusion

Given that botulinum toxin is so potent, and that uptake begins in the stomach and proceeds at a rapid rate, it is not surprising that diarrhea does not seem to have much of an effect on outcomes. These recently discovered mechanisms may explain the lack of significant results in this thesis study other than the association of diarrhea with fatigue. Diarrhea can be a very fatiguing event in any case, whatever the cause. It is interesting that frequencies of neurologic symptoms are less in those cases with diarrhea. That trend may have achieved statistical significance in a larger cohort. Without a large enough study population, this question cannot be answered decisively. The question could be best answered in the future by combining data from outbreaks across the U.S. over a period of years to achieve an adequate sample size.

APPENDICES

Appendix A

348 ELA, Political Science Department
Southwest Texas State University
San Marcos, Texas 78666

Dear Dr. Charles P. Garofalo

Peter Wolf, Mt(ASCP), a Master of Science candidate at Southwest Texas State University, has requested permission to use data from the Texas Department of Health, Infectious Disease Epidemiology & Surveillance Division for his thesis. I have read and approve of his proposal to study the Epidemiology and Clinical Aspects of Foodborne Botulism in Texas, 1992-2001. Therefore, I, Kate Hendricks, M.D., grant him/her permission to use our data from the El Paso and Dallas Botulism outbreak files for this purpose. Peter Wolf is permitted to access data with personal identifiers but will sign a confidentiality agreement with the Texas Department of Health. Peter Wolf will be permitted to remove data with identifiers from the TDH data repository under the condition that the data will be kept in a secured location for the duration of the study and will be returned to TDH at the conclusion of the study (ie, when the study manuscript has been written or at the end of two years whichever comes first). This refers to electronic as well as hard copies of data.

The Epidemiology and Clinical Aspects of Foodborne Botulism in Texas, 1992-2001, has previously been approved by the TDH Institutional Review Board for the protection of human subjects. If you need any additional information, please feel free to contact me at 512/458-7676.

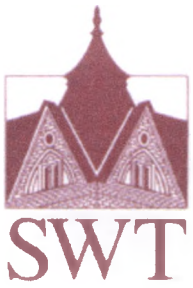
Sincerely,

K Hendricks

Kate Hendricks, M.D.
Director, Infectious Disease Epidemiology & Surveillance Division

8/2/200210:0 AM Marilyn Felkner response to data request

Appendix B



Institutional Review Board

Certification of Review and Approval by the Southwest Texas State University Institutional Review Board

IRB Reference Number
02-0218

The project titled:

The Epidemiology and Clinical Aspects of Foodborne Botulism in Texas, 1992-2001

by Peter Wolf under the supervision of Jean Brender

has been APPROVED, effective 10/1/2002.

The Southwest Texas Institutional Review Board shall conduct continuing review of this research appropriate to the degree of risk and the length of the project period, but not less than once per year.

Charles Garofalo
Chair, Institutional Review Board

Billy C. Covington
Associate Vice President, Office of Sponsored Programs/
Director, Federal Relations

Southwest Texas State University
601 University Drive San Marcos, Texas 78666-4605
512-245-2414
SWT is a member of the Texas State University System.

Appendix C

Logistic Regression of Hospitalized

Case Processing Summary

Unweighted Cases[a]		N	Percent
Selected Cases	Included in Analysis	43	100.0
	Missing Cases	0	0
	Total	43	100.0
Unselected Cases		0	.0
Total		43	100.0

a. If weight is in effect, see classification table for the total number of cases.

Dependent Variable Encoding

Original Value	Internal Value
no	0
yes	1

Categorical Variables Codings

		Frequency	Parameter (1)
SEX	male	25	.500
	female	18	-.500

Block 0: Beginning Block

Iteration History[a,b,c]

Iteration		-2 Log likelihood	Coefficients Constant
Step 0	1	50.956	.884
	2	50.918	.948
	3	50.918	.949

a. Constant is included in the model.

b. Initial -2 Log Likelihood: 50.918

c. Estimation terminated at iteration number 3 because parameter estimates changed by less than .001.

Classification Table[a,b]

			Predicted	
			HOSPITAL__	
	Observed		no	yes
Step 0	HOSPITAL__	no	0	12
		yes	0	31
	Overall Percentage			

Classification Table[a,b]

Observed			Predicted
			Percentage Correct
Step 0	HOSPITAL__	no	.0
		yes	100.0
	Overall Percentage		72.1

a. Constant is included in the model.

b. The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df
Step 0	Constant	.949	.340	7.793	1

Variables in the Equation

		Sig.	Exp(B)
Step 0	Constant	.005	2.583

Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	DIARRHEA	.453	1	.501
		AGE_____	6.937	1	.008
		SEX_____(1)	1.944	1	.163
	Overall Statistics		7.690	3	.053

Block 1: Method = Enter

Iteration History[a,b,c,d]

Iteration		-2 Log likelihood	Coefficients			
			Constant	DIARRHEA	AGE	SEX (1)
Step 1	1	43.636	2.393	- 107	-.038	-.491
	2	42.718	3.254	-.230	-.050	-.764
	3	42.688	3.452	-.272	-.052	-.842
	4	42.688	3.461	- 274	-.052	-.846

a. Method: Enter

b. Constant is included in the model.

c. Initial -2 Log Likelihood: 50.918

d. Estimation terminated at iteration number 4 because log-likelihood decreased by less than .010 percent.

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	8.230	3	.041
	Block	8.230	3	.041
	Model	8.230	3	.041

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	42.688	.174	.251

Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	6.734	8	.566

Contingency Table for Hosmer and Lemeshow Test

		HOSPITAL = no		HOSPITAL = yes		Total
		Observed	Expected	Observed	Expected	
Step 1	1	2	2.752	2	1.248	4
	2	3	2.029	1	1.971	4
	3	2	1 693	2	2.307	4
	4	1	1.434	3	2.566	4
	5	0	1.079	4	2.921	4
	6	2	.926	2	3.074	4
	7	1	.720	3	3.280	4
	8	0	.492	4	3.508	4
	9	0	.395	4	3.605	4
	10	1	.481	6	6.519	7

Table 2
Logistic Regression Results of Age, Sex,, and Diarrhea as Predictors for Hospitalization, Botulism cases

		B	S E	Wald	df	Sig.	Exp(B)
Step 1[a]	DIARRHEA	- 274	790	120	1	.729	.760
	AGE	-.052	.024	4.745	1	.029	.949
	SEX	-.846	.845	1.002	1	317	.429
	Constant	3.461	1.485	5.434	1	.020	31.856

Table 2
Logistic Regression Results of Age, Sex,, and Diarrhea as Predictors for Hospitalization, Botulism cases

		95.0% C.I.for EXP(B)	
		Lower	Upper
Step 1[a]	DIARRHEA	162	3.577
	AGE	.906	.995
	SEX	.082	2.248
	Constant		

a. Variable(s) entered on step 1 DIARRHEA, AGE_____, SEX_____.

Correlation Matrix

		Constant	DIARRHEA	AGE_____	SEX_____(1)
Step 1	Constant	1.000	-.714	-.530	-.266
	DIARRHEA	-.714	1.000	-.149	.261
	AGE_____	-.530	-.149	1.000	-.071
	SEX_____(1)	-.266	.261	-.071	1.000

Logistic Regression of Intubated

Case Processing Summary

Unweighted Cases[a]		N	Percent
Selected Cases	Included in Analysis	43	100.0
	Missing Cases	0	.0
	Total	43	100.0
Unselected Cases		0	.0
Total		43	100.0

a. If weight is in effect, see classification table for the total number of cases.

Dependent Variable Encoding

Original Value	Internal Value
1	0
2	1

Categorical Variables Codings

		Frequency	Parameter (1)
SEX_____	1	25	-.500
	2	18	.500
DIARRHEA__	1	25	-.500
	2	18	.500

Block 0: Beginning Block

Classification Table[a,b]

			Predicted	
			INTUBATED	
	Observed		no	yes
Step 0	INTUBATED	no	34	0
		yes	9	0
	Overall Percentage			

Classification Table[a,b]

Observed			Predicted: Percentage Correct
Step 0	INTUBATED	no	100.0
		yes	.0
	Overall Percentage		79.1

a. Constant is included in the model.

b. The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df
Step 0	Constant	-1.329	.375	12.571	1

Variables in the Equation

		Sig.	Exp(B)
Step 0	Constant	.000	.265

Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	DIARRHEA(1)	.877	1	.349
		AGE_____	1.412	1	.235
		SEX_____(1)	.031	1	.860
	Overall Statistics		2.946	3	.400

Block 1: Method = Enter

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	3.018	3	.389
	Block	3.018	3	.389
	Model	3.018	3	.389

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	41.103	.068	.106

Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	4.668	8	.792

Contingency Table for Hosmer and Lemeshow Test

		INTUBATED = no		INTUBATED = yes		
		Observed	Expected	Observed	Expected	Total
Step 1	1	4	3.739	0	.261	4
	2	3	3.622	1	.378	4
	3	3	3.412	1	.588	4
	4	5	4.206	0	.794	5
	5	3	3.327	1	.673	4
	6	3	3.284	1	.716	4
	7	4	3.230	0	.770	4
	8	3	2.979	1	1.021	4
	9	2	2.686	2	1.314	4
	10	4	3.516	2	2.484	6

Classification Table[a]

			Predicted	
			INTUBATED	
	Observed		no	yes
Step 1	INTUBATED	no	34	0
		yes	9	0
	Overall Percentage			

Classification Table[a]

Observed			Predicted: Percentage Correct
Step 1	INTUBATED	no	100.0
		yes	.0
	Overall Percentage		79.1

a. The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df	Sig.
Step 1[a]	DIARRHEA(1)	.981	.805	1.483	1	.223
	AGE_____	-.035	.025	1.935	1	.164
	SEX_____(1)	-.045	.787	.003	1	.955
	Constant	-.166	.851	.038	1	.845

Variables in the Equation

		Exp(B)	95.0% C.I.for EXP(B)	
			Lower	Upper
Step 1[a]	DIARRHEA(1)	2.667	.550	12.930
	AGE_____	.966	.920	1.014
	SEX_____(1)	.956	.204	4.475
	Constant	847		

a. Variable(s) entered on step 1: DIARRHEA, AGE_____, SEX_____.

Correlation Matrix

		Constant	DIARRHEA(1)	AGE_____	SEX_____(1)
Step 1	Constant	1.000	.216	-.888	-.047
	DIARRHEA(1)	216	1.000	-.249	.038
	AGE_____	-.888	-.249	1.000	.101
	SEX_____(1)	-.047	.038	.101	1.000

Appendix D

Multivariate Analysis of Covariance (ANCOVA)

Between-Subjects Factors

		Value Label	N
DIARRHEA__	1	no	25
	2	yes	18

Box's Test of Equality of Covariance Matrices[a]

Box's M	17.715
F	1.018
df1	15
df2	5362.266
Sig	.432

Tests the null hypothesis that the observed covariance matrices of the dependent variables are equal across groups.

a. Design: Intercept+AGE_____+SEX_____+DIARRHEA

Table 3
Multivariate Tests of Effects of Age, Sex and Diarrhea on Symptoms and Outcomes

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.241	2 223	5.000	35 000	.074
	Wilks' Lambda	.759	2.223	5.000	35 000	074
	Hotelling's Trace	.318	2.223	5.000	35 000	.074
	Roy's Largest Root	.318	2.223	5 000	35.000	.074
AGE_____	Pillai's Trace	.254	2.389	5.000	35 000	.058
	Wilks' Lambda	.746	2 389	5 000	35 000	.058
	Hotelling's Trace	.341	2 389	5.000	35.000	058
	Roy's Largest Root	.341	2.389	5.000	35.000	058
SEX_____	Pillai's Trace	.096	.741	5.000	35.000	.598
	Wilks' Lambda	.904	.741	5.000	35.000	598
	Hotelling's Trace	.106	.741	5.000	35.000	598
	Roy's Largest Root	.106	.741	5 000	35 000	.598
DIARRHEA	Pillai's Trace	.126	1.012	5.000	35.000	.425
	Wilks' Lambda	.874	1.012	5.000	35.000	.425
	Hotelling's Trace	.145	1 012	5 000	35 000	.425
	Roy's Largest Root	.145	1.012	5.000	35.000	.425

Table 3 Multivariate Tests of Effects of Age, Sex and Diarrhea on Symptoms and Outcomes [b]

Effect		Value	F	Hypothesis	Error df	Sig
Intercept	Pillai's Trace	24	2 22	5 00	35 0(	07
	Wilks' Lambda	75	2 22	5 00	35 0(	07
	Hotelling's Trace	31	2 22	5 00	35 0(	07
	Roy's Largest Root	31	2 22	5 00	35 0(	07
AGE_____	Pillai's Trace	25	2 38	5 00	35 0(	05
	Wilks' Lambda	74	2 38	5 00	35 0(	05
	Hotelling's Trace	34	2 38	5 00	35 0(	05
	Roy's Largest Root	34	2 38	5 00	35 0(	05
SEX_____	Pillai's Trace	09	74	5 00	35.0(	59
	Wilks' Lambda	90	74	5 00	35 0(	.59
	Hotelling's Trace	10	74	5.00	35 0(	.59
	Roy's Largest Root	10	74	5 00	35 0(	59
DIARRHEA	Pillai's Trace	12	1 01	5 00	35 0(	42
	Wilks' Lambda	87	1 01	5.00	35 0(	42
	Hotelling's Trace	.14	1.01	5 00	35 0(	.42
	Roy's Largest Root	14	1 01	5 00	35 0(	42

[a] Exact statistic

[b] Design Intercept+AGE_____+SEX_____+DIARRHEA

Levene's Test of Equality of Error Variances[a]

	F	df1	df2	Sig.
vision/2	.145	1	41	.706
oral/3	.069	1	41	.794
intervention/2	.691	1	41	.411
neurological/6	1.351	1	41	.252
total/12	.808	1	41	.374

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept+AGE_____+SEX_____+DIARRHEA

Table 4 Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	vision/2	.247[a]	3	8.242E-02	.409	747
	oral/3	710[b]	3	.237	1.742	.174
	intervention/2	.721[c]	3	.240	2.238	.099
	neurological/6	.433[d]	3	.144	1.375	.265
	total/12	.469[e]	3	.156	2.094	.117

Table 4 Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	vision/2	.869	1	.869	4.312	.044
	oral/3	4.563E-02	1	4.563E-02	336	.565
	intervention/2	.856	1	.856	7 972	.007
	neurological/6	.199	1	.199	1.891	177
	total/12	.335	1	335	4.492	.040
AGE_____	vision/2	3.089E-02	1	3.089E-02	.153	.697
	oral/3	.155	1	.155	1.141	.292
	intervention/2	.545	1	545	5.079	030
	neurological/6	1.237E-02	1	1.237E-02	.118	.733
	total/12	2 893E-02	1	2 893E-02	.388	537
SEX_____	vision/2	5 863E-03	1	5 863E-03	029	865
	oral/3	.412	1	.412	3.032	090
	intervention/2	5.503E-02	1	5.503E-02	.513	478
	neurological/6	.189	1	.189	1.802	187
	total/12	.176	1	176	2.361	132
DIARRHEA	vision/2	.223	1	.223	1 106	299
	oral/3	.126	1	.126	928	341
	intervention/2	1.080E-02	1	1 080E-02	.101	.753
	neurological/6	190	1	.190	1.810	.186
	total/12	.223	1	223	2 986	.092
Error	vision/2	7 857	39	201		
	oral/3	5 295	39	136		
	intervention/2	4 186	39	.107		
	neurological/6	4.094	39	.105		
	total/12	2.909	39	7.460E-02		
Total	vision/2	21.500	43			
	oral/3	18 667	43			
	intervention/2	13.750	43			
	neurological/6	15 444	43			
	total/12	13.715	43			
Corrected Total	vision/2	8 105	42			
	oral/3	6 005	42			
	intervention/2	4.907	42			
	neurological/6	4.527	42			
	total/12	3.378	42			

a. R Squared = 031 (Adjusted R Squared = - 044)

b R Squared = .118 (Adjusted R Squared = .050)

c R Squared = 147 (Adjusted R Squared = 081)

d R Squared = 096 (Adjusted R Squared = 026)

e R Squared = 139 (Adjusted R Squared = 072)

Parameter Estimates

Dependent Variable	Parameter	B	Std. Error	t	Sig.
vision/2	Intercept	.667	.309	2.158	.037
	AGE_____	-1.6E-03	.004	-.392	.697
	SEX_____	2.43E-02	.143	.171	865
	[DIARRHEA=1]	-.150	.143	-1.052	.299
	[DIARRHEA=2]	0[a]	.	.	.
oral/3	Intercept	.192	.254	.757	.454
	AGE_____	3.61E-03	.003	1.068	.292
	SEX_____	.204	.117	1.741	.090
	[DIARRHEA=1]	-.113	.117	-.963	.341
	[DIARRHEA=2]	0[a]	.	.	
intervention/2	Intercept	.604	.226	2.677	.011
	AGE_____	-6.8E-03	.003	-2.254	.030
	SEX_____	7.46E-02	.104	.716	.478
	[DIARRHEA=1]	-3.3E-02	.104	-.317	.753
	[DIARRHEA=2]	0[a]	.	.	.
neurological/6	Intercept	.353	.223	1.579	.122
	AGE_____	1.02E-03	.003	.343	.733
	SEX_____	.138	.103	1.342	187
	[DIARRHEA=1]	-.139	.103	-1.345	.186
	[DIARRHEA=2]	0[a]	.	.	.
total/12	Intercept	.443	188	2.354	.024
	AGE_____	-1.6E-03	.003	-.623	.537
	SEX_____	.133	.087	1.537	.132
	[DIARRHEA=1]	-.150	.087	-1.728	.092
	[DIARRHEA=2]	0[a]	.	.	.

Parameter Estimates

Dependent Variable	Parameter	95% Confidence Interval	
		Lower Bound	Upper Bound
vision/2	Intercept	4.183E-02	1.293
	AGE_____	-9 949E-03	6.72E-03
	SEX_____	-.264	.313
	[DIARRHEA=1]	-.439	.139
	[DIARRHEA=2]	.	.
oral/3	Intercept	-.321	.706
	AGE_____	-3.230E-03	1.05E-02
	SEX_____	-3.298E-02	.441
	[DIARRHEA=1]	-.350	.124
	[DIARRHEA=2]	.	.
intervention/2	Intercept	.148	1.061
	AGE_____	-1.286E-02	-6.9E-04
	SEX_____	-.136	.285
	[DIARRHEA=1]	-.244	.178
	[DIARRHEA=2]	.	.
neurological/6	Intercept	-9.910E-02	.804
	AGE_____	-4.996E-03	7.04E-03
	SEX_____	-7 010E-02	.347
	[DIARRHEA=1]	-.347	6.98E-02
	[DIARRHEA=2]		.
total/12	Intercept	6.230E-02	.824
	AGE_____	-6.634E-03	3.51E-03
	SEX_____	-4.222E-02	.309
	[DIARRHEA=1]	-.326	2.56E-02
	[DIARRHEA=2]	.	.

a. This parameter is set to zero because it is redundant.

Lack of Fit

Multivariate Tests

Dependent Variables	Multivariate Tests	Statistics		
		Value	F	Hypothesis df
vision/2, oral/3, intervention/2, neurological/6, total/12	Pillai's Trace	4.508	1.347	170.000
	Wilks' Lambda	.000	1.828	170.000
	Hotelling's Trace	.	.	170.000
	Roy's Largest Root	943.067	138.686[a]	34.000
vision/2, oral/3, intervention/2, neurological/6	Pillai's Trace	3.522	1.084	136.000
	Wilks' Lambda	.000	1.001	136.000
	Hotelling's Trace	94.567	.348	136.000
	Roy's Largest Root	66.812	9.825[a]	34.000
vision/2, oral/3, intervention/2, total/12	Pillai's Trace	3.627	1.431	136.000
	Wilks' Lambda	.000	1 318	136.000
	Hotelling's Trace	114.162	.420	136.000
	Roy's Largest Root	77.794	11.440[a]	34.000
vision/2, oral/3, neurological/6, total/12	Pillai's Trace	3.583	1.263	136.000
	Wilks' Lambda	000	1.232	136.000
	Hotelling's Trace	153.083	.563	136.000
	Roy's Largest Root	126.431	18.593[a]	34.000
vision/2, intervention/2, neurological/6, total/12	Pillai's Trace	3.629	1.438	136.000
	Wilks' Lambda	.000	1.756	136.000
	Hotelling's Trace	189.399	.696	136.000
	Roy's Largest Root	115.617	17.002[a]	34.000
oral/3, intervention/2, neurological/6, total/12	Pillai's Trace	3.667	1 618	136.000
	Wilks' Lambda	.000	2.358	136.000
	Hotelling's Trace	306.301	1.126	136.000
	Roy's Largest Root	230.027	33.828[a]	34.000
vision/2, oral/3, intervention/2	Pillai's Trace	2.673	1.204	102.000
	Wilks' Lambda	.000	1.357	102.000
	Hotelling's Trace	71.915	1.175	102.000
	Roy's Largest Root	55.834	8.211[a]	34.000
vision/2, oral/3, neurological/6	Pillai's Trace	2.624	1.027	102.000
	Wilks' Lambda	.001	.802	102.000
	Hotelling's Trace	30 019	.491	102.000
	Roy's Largest Root	19.424	2.857[a]	34.000
vision/2, oral/3, total/12	Pillai's Trace	2.696	1.304	102.000
	Wilks' Lambda	.001	1.062	102.000
	Hotelling's Trace	39 087	.639	102.000
	Roy's Largest Root	21.996	3.235[a]	34.000
vision/2, intervention/2, neurological/6	Pillai's Trace	2.644	1.092	102.000
	Wilks' Lambda	.000	1.269	102.000
	Hotelling's Trace	78.507	1.283	102.000
	Roy's Largest Root	66.603	9.795[a]	34.000

Multivariate Tests

Dependent Variables	Multivariate Tests	Statistics		
		Value	F	Hypothesis df
vision/2, intervention/2, total/12	Pillai's Trace	2.681	1.236	102.000
	Wilks' Lambda	.000	1 552	102.000
	Hotelling's Trace	94.589	1.546	102.000
	Roy's Largest Root	77.723	11.430[a]	34.000
vision/2, neurological/6, total/12	Pillai's Trace	2.696	1 306	102.000
	Wilks' Lambda	.000	1.401	102.000
	Hotelling's Trace	83.055	1.357	102.000
	Roy's Largest Root	70.279	10.335[a]	34.000
oral/3, intervention/2, neurological/6	Pillai's Trace	2.679	1.227	102.000
	Wilks' Lambda	.000	1 500	102.000
	Hotelling's Trace	78.874	1.289	102 000
	Roy's Largest Root	58.289	8.572[a]	34.000
oral/3, intervention/2, total/12	Pillai's Trace	2.690	1.276	102.000
	Wilks' Lambda	000	1.543	102 000
	Hotelling's Trace	85.954	1.404	102.000
	Roy's Largest Root	67.363	9.906[a]	34.000
oral/3, neurological/6, total/12	Pillai's Trace	2.740	1 548	102.000
	Wilks' Lambda	.000	2.045	102.000
	Hotelling's Trace	141.767	2.316	102.000
	Roy's Largest Root	122.034	17.946[a]	34 000
intervention/2, neurological/6, total/12	Pillai's Trace	2.736	1.524	102.000
	Wilks' Lambda	.000	2.648	102.000
	Hotelling's Trace	161.897	2 645	102.000
	Roy's Largest Root	114.655	16.861[a]	34.000
vision/2, oral/3	Pillai's Trace	1.778	1.180	68.000
	Wilks' Lambda	.011	1.017[b]	68.000
	Hotelling's Trace	18.611	.821	68.000
	Roy's Largest Root	12.936	1.902[a]	34.000
vision/2, intervention/2	Pillai's Trace	1.729	.937	68.000
	Wilks' Lambda	.008	1.186[b]	68.000
	Hotelling's Trace	31.286	1.380	68.000
	Roy's Largest Root	28.064	4 127[a]	34 000
vision/2, neurological/6	Pillai's Trace	1.740	.986	68.000
	Wilks' Lambda	.016	.810[b]	68.000
	Hotelling's Trace	14.150	.624	68.000
	Roy's Largest Root	8.807	1.295[a]	34.000
vision/2, total/12	Pillai's Trace	1.754	1.049	68.000
	Wilks' Lambda	.012	.956[b]	68.000
	Hotelling's Trace	18.490	.816	68.000
	Roy's Largest Root	13.898	2.044[a]	34.000
oral/3, intervention/2	Pillai's Trace	1.747	1.014	68.000
	Wilks' Lambda	.009	1.143[b]	68.000
	Hotelling's Trace	27.070	1.194	68.000
	Roy's Largest Root	23.359	3.435[a]	34.000

Multivariate Tests

Dependent Variables	Multivariate Tests	Statistics		
		Value	F	Hypothesis df
oral/3, neurological/6	Pillai's Trace	1.780	1.190	68.000
	Wilks' Lambda	.009	1.133[b]	68.000
	Hotelling's Trace	22.878	1.009	68.000
	Roy's Largest Root	17.894	2.632[a]	34.000
oral/3, total/12	Pillai's Trace	1.754	1.048	68.000
	Wilks' Lambda	.011	.993[b]	68.000
	Hotelling's Trace	19.936	.880	68.000
	Roy's Largest Root	15.548	2.286[a]	34.000
intervention/2, neurological/6	Pillai's Trace	1.746	1.012	68.000
	Wilks' Lambda	.005	1.583[b]	68.000
	Hotelling's Trace	51.005	2.250	68.000
	Roy's Largest Root	47.715	7.017[a]	34.000
intervention/2, total/12	Pillai's Trace	1.757	1.063	68.000
	Wilks' Lambda	.004	1.866[b]	68.000
	Hotelling's Trace	67.087	2.960	68.000
	Roy's Largest Root	63.694	9.367[a]	34.000
neurological/6, total/12	Pillai's Trace	1.808	1.387	68.000
	Wilks' Lambda	.003	1.967[b]	68.000
	Hotelling's Trace	58.198	2.568	68.000
	Roy's Largest Root	53.428	7.857[a]	34.000
vision/2	Pillai's Trace	.857	.880[b]	34.000
	Wilks' Lambda	.143	.880[b]	34.000
	Hotelling's Trace	5.984	.880[b]	34.000
	Roy's Largest Root	5.984	.880[b]	34.000
oral/3	Pillai's Trace	.895	1.255[b]	34.000
	Wilks' Lambda	.105	1.255[b]	34.000
	Hotelling's Trace	8.532	1.255[b]	34.000
	Roy's Largest Root	8.532	1.255[b]	34.000
intervention/2	Pillai's Trace	.791	.557[b]	34 000
	Wilks' Lambda	.209	.557[b]	34.000
	Hotelling's Trace	3.784	.557[b]	34.000
	Roy's Largest Root	3.784	.557[b]	34.000
neurological/6	Pillai's Trace	.844	.795[b]	34.000
	Wilks' Lambda	.156	.795[b]	34.000
	Hotelling's Trace	5 408	.795[b]	34.000
	Roy's Largest Root	5.408	.795[b]	34.000
total/12	Pillai's Trace	.827	.703[b]	34.000
	Wilks' Lambda	.173	.703[b]	34.000
	Hotelling's Trace	4.779	.703[b]	34.000
	Roy's Largest Root	4.779	.703[b]	34.000

Multivariate Tests

Dependent Variables	Multivariate Tests	Statistics	
		Error df	Sig.
vision/2, oral/3, intervention/2, neurological/6, total/12	Pillai's Trace	25.000	.192
	Wilks' Lambda	10.182	.143
	Hotelling's Trace	.	.
	Roy's Largest Root	5.000	.000
vision/2, oral/3, intervention/2, neurological/6	Pillai's Trace	20.000	.441
	Wilks' Lambda	10.623	.551
	Hotelling's Trace	2.000	.940
	Roy's Largest Root	5 000	.009
vision/2, oral/3, intervention/2, total/12	Pillai's Trace	20.000	.178
	Wilks' Lambda	10.623	.324
	Hotelling's Trace	2.000	.904
	Roy's Largest Root	5.000	.006
vision/2, oral/3, neurological/6, total/12	Pillai's Trace	20.000	.280
	Wilks' Lambda	10.623	.375
	Hotelling's Trace	2.000	.827
	Roy's Largest Root	5.000	.002
vision/2, intervention/2, neurological/6, total/12	Pillai's Trace	20.000	.175
	Wilks' Lambda	10.623	.154
	Hotelling's Trace	2.000	.759
	Roy's Largest Root	5.000	.002
oral/3, intervention/2, neurological/6, total/12	Pillai's Trace	20.000	.106
	Wilks' Lambda	10.623	.060
	Hotelling's Trace	2.000	.586
	Roy's Largest Root	5.000	.000
vision/2, oral/3, intervention/2	Pillai's Trace	15.000	.358
	Wilks' Lambda	9.885	.313
	Hotelling's Trace	5.000	.483
	Roy's Largest Root	5.000	.013
vision/2, oral/3, neurological/6	Pillai's Trace	15.000	.512
	Wilks' Lambda	9.885	.729
	Hotelling's Trace	5.000	.921
	Roy's Largest Root	5.000	.121
vision/2, oral/3, total/12	Pillai's Trace	15.000	.290
	Wilks' Lambda	9.885	.502
	Hotelling's Trace	5.000	.824
	Roy's Largest Root	5.000	.096
vision/2, intervention/2, neurological/6	Pillai's Trace	15.000	.450
	Wilks' Lambda	9.885	.361
	Hotelling's Trace	5.000	.433
	Roy's Largest Root	5.000	.009

Multivariate Tests

Dependent Variables	Multivariate Tests	Statistics	
		Error df	Sig.
vision/2, intervention/2, total/12	Pillai's Trace	15.000	.335
	Wilks' Lambda	9.885	.229
	Hotelling's Trace	5.000	.336
	Roy's Largest Root	5.000	.006
vision/2, neurological/6, total/12	Pillai's Trace	15.000	.288
	Wilks' Lambda	9.885	.292
	Hotelling's Trace	5.000	.402
	Roy's Largest Root	5.000	.008
oral/3, intervention/2, neurological/6	Pillai's Trace	15.000	.341
	Wilks' Lambda	9 885	.249
	Hotelling's Trace	5.000	.431
	Roy's Largest Root	5.000	.012
oral/3, intervention/2, total/12	Pillai's Trace	15.000	.308
	Wilks' Lambda	9 885	.233
	Hotelling's Trace	5.000	.384
	Roy's Largest Root	5.000	.009
oral/3, neurological/6, total/12	Pillai's Trace	15.000	.170
	Wilks' Lambda	9.885	.108
	Hotelling's Trace	5.000	.174
	Roy's Largest Root	5.000	.002
intervention/2, neurological/6, total/12	Pillai's Trace	15.000	.179
	Wilks' Lambda	9.885	.047
	Hotelling's Trace	5 000	.137
	Roy's Largest Root	5.000	.003
vision/2, oral/3	Pillai's Trace	10.000	.414
	Wilks' Lambda	8.000	.543
	Hotelling's Trace	6.000	.692
	Roy's Largest Root	5.000	.245
vision/2, intervention/2	Pillai's Trace	10.000	.600
	Wilks' Lambda	8.000	.432
	Hotelling's Trace	6.000	.369
	Roy's Largest Root	5.000	.059
vision/2, neurological/6	Pillai's Trace	10.000	.559
	Wilks' Lambda	8.000	.707
	Hotelling's Trace	6.000	.840
	Roy's Largest Root	5.000	.424
vision/2, total/12	Pillai's Trace	10.000	.509
	Wilks' Lambda	8.000	.589
	Hotelling's Trace	6.000	.696
	Roy's Largest Root	5.000	.218
oral/3, intervention/2	Pillai's Trace	10.000	.536
	Wilks' Lambda	8.000	.458
	Hotelling's Trace	6.000	.455
	Roy's Largest Root	5.000	.085

Multivariate Tests

Dependent Variables	Multivariate Tests	Statistics	
		Error df	Sig.
oral/3, neurological/6	Pillai's Trace	10.000	.408
	Wilks' Lambda	8.000	.464
	Hotelling's Trace	6.000	.561
	Roy's Largest Root	5.000	.141
oral/3, total/12	Pillai's Trace	10.000	.509
	Wilks' Lambda	8.000	.561
	Hotelling's Trace	6.000	.649
	Roy's Largest Root	5.000	.181
intervention/2, neurological/6	Pillai's Trace	10.000	.537
	Wilks' Lambda	8.000	.252
	Hotelling's Trace	6.000	.154
	Roy's Largest Root	5.000	.019
intervention/2, total/12	Pillai's Trace	10.000	.498
	Wilks' Lambda	8.000	.175
	Hotelling's Trace	6.000	.085
	Roy's Largest Root	5.000	.010
neurological/6, total/12	Pillai's Trace	10.000	.298
	Wilks' Lambda	8.000	.154
	Hotelling's Trace	6.000	.117
	Roy's Largest Root	5.000	.015
vision/2	Pillai's Trace	5.000	.640
	Wilks' Lambda	5.000	.640
	Hotelling's Trace	5.000	.640
	Roy's Largest Root	5.000	.640
oral/3	Pillai's Trace	5.000	.441
	Wilks' Lambda	5.000	.441
	Hotelling's Trace	5.000	.441
	Roy's Largest Root	5.000	.441
intervention/2	Pillai's Trace	5.000	.860
	Wilks' Lambda	5.000	.860
	Hotelling's Trace	5.000	.860
	Roy's Largest Root	5.000	.860
neurological/6	Pillai's Trace	5.000	.695
	Wilks' Lambda	5.000	.695
	Hotelling's Trace	5.000	.695
	Roy's Largest Root	5.000	.695
total/12	Pillai's Trace	5 000	.759
	Wilks' Lambda	5.000	.759
	Hotelling's Trace	5.000	.759
	Roy's Largest Root	5.000	.759

a. The statistic is an upper bound on F that yields a lower bound on the significance level.

b. Exact statistic

Univariate Tests

Dependent Variable	Source	Sum of Squares	df	Mean Square	F	Sig.
vision/2	Lack of Fit	6.732	34	.198	.880	.640
	Pure Error	1.125	5	.225		
oral/3	Lack of Fit	4.740	34	.139	1.255	.441
	Pure Error	.556	5	.111		
intervention/2	Lack of Fit	3.311	34	.097	.557	.860
	Pure Error	.875	5	.175		
neurological/6	Lack of Fit	3.455	34	.102	.795	.695
	Pure Error	.639	5	.128		
total/12	Lack of Fit	2.406	34	.071	.703	.759
	Pure Error	.503	5	.101		

References

Abramson, J. H. & Gahlinger, P.M. (2000). Computer Programs for Epidemiologists - PEPI, Version 3.01, Stone Mountain, Georgia: USD Inc.

Angulo F. J., Getz J., Taylor J. P., Hendricks K. A., Hatheway C. L., Barth S. S., Solomon H. M., Larson A. E., Johnson E. A., Nickey L. N. & Ries A. A. (1998). A Large Outbreak of Botulism: The Hazardous Baked Potato. Journal of Infectious Disease, 178, 172-177.

Arnon S. S., Schechter R. & The Working Group on Civilian Biodefense. (2001). Medical and Public Health Management Following the Use of a Biological Weapon: Consensus Statements of The Working Group on Civilian Biodefense. JAMA, 285, 1059-1070

Botulism, disease information, NCID, CDC. (2001) [On-line]. Available: http://www.cdc.gov/ncidod/diseases/submenus/sub_botulism.htm

Centers for Disease Control (CDC). (1997). Case Definitions for Infectious Conditions Under Public Health Surveillance MMWR, 46, 1-55

Centers for Disease Control (CDC). (1998). Botulism in the United States 1899-1996 Handbook for Epidemiologists, Clinicians and Laboratory Workers.

Centers for Disease Control (CDC). (2002). CDC Health Topic: Botulism

Erbguth, F. J. & Naumann, M. (1999). Historical aspects of botulinum toxin, Justinus Kerner (1786-1862) and the "sausage poison". Neurology, 53, 1850-1853

Hair J.F., Anderson R.E., Tatham R.L. & Black W.C. (1998). Multivariate Data Analysis 5th Edition. Chapters 5 & 6. New Jersey: Prentice Hall.

Kalluri, P. (2001). Outbreak of Foodborne Botulism 2001, North Texas (Presentation). Centers for Disease Control and Prevention

Maksymowych A. B., Reinhard M., Malizio C. J., Goodnough M., Johnson E. A., & Simpson L.L. (1999). Pure Botulinum Neurotoxin Is Absorbed from the Stomach and

Small Intestine and Produces Peripheral Neuromuscular Blockade. Infection and Immunity, 67, 4708-4712.

Maksymowych A. B. & Simpson L. L. (1998). Binding and Transcytosis of Botulinum Neurotoxin by Polarized Human Colon Carcinoma Cells. The Journal of Biological Chemistry, 273, 21950–21957.

Solomon, H. M. & Lilly, T. Jr. (1998). Bacteriological Analytical Manual, 8th Edition, Chapter 17. U.S. Food & Drug Administration

SPSS Incorporated. (2001). SPSS® Base 11.0. Chicago, Illinois.

Texas Department of Health (TDH). (1995). Foodborne Botulism Outbreak in El Paso. Disease Prevention News (DPN), 55, 1-2

Texas Department of Health files on Botulism

Todar, K. (2002). The Pathogenic Clostridia. University of Wisconsin-Madison Department of Bacteriology [On-line]. Available: http://www.bact.wisc.edu/bact330/bact330homepage

VITA

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