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Outcome of Adult Horses with Botulism Treated at a Veterinary Hospital: 92 Cases (1989–2013)

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Background: There are no studies evaluating a large population of adult horses treated for botulism. Reported survival rates in outbreak situations are low; however, many horses in outbreaks do not receive treatment.

Hypothesis/Objectives: That adult horses treated at a veterinary hospital would have improved survival compared to outbreak situations. Additional aims included identification of predictors of nonsurvival.

Animals: All horses greater than 6 months of age with a final diagnosis of botulism admitted to a veterinary teaching hospital between 1989 and 2013 were included.

Methods: Retrospective study. Historical, admission, and hospitalization data were retrieved from medical records and associations between variables and nonsurvival were identified using logistic regression. Two multivariable models were developed pertaining to (1) information available at admission and (2) clinical findings during hospitalization.

Results: Ninety-two records met inclusion criteria. Retained variables for the two models indicated that higher rectal temperature (OR, 1.94; CI, 1.19–3.17) and dysphagia (OR, 4.04; CI, 1.01–16.17) observed at admission increased the odds of survival, as did treatment with antitoxin (OR, 121.30; CI, 9.94–1,480.65). Horses with abnormal respiratory effort or inability to stand had decreased odds of survival. Overall survival was 48% but was significantly higher (67%, $P = .011$) for horses that arrived standing, and even higher (95%, $P < .001$) for horses that remained able to stand throughout hospitalization. Complications occurred in 62% of horses but were not associated with nonsurvival.

Conclusions and Clinical Importance: Horses that lose the ability to stand have a poor chance of survival. Complications are common in treated horses but do not reduce survival.

Key words: Botulinum; Clostridium; Neurotoxin; Recumbency.

Botulism, caused by the *Clostridium botulinum* neurotoxin, is a disease characterized by progressive flaccid paresis and cranial nerve deficits, particularly dysphagia. Horses are most commonly affected by type B botulism, which in adult horses is acquired primarily through ingestion of preformed toxin with feed.[1] Disease course is related to total toxin exposure but most commonly results in death unless the horse is treated promptly with specific antitoxin.[1]

There have been no large retrospective studies on botulism in adult horses that are hospitalized. Most reports detail outbreak situations, which tend to have high case fatality and limited treatment efforts. The survival rate has ranged from approximately 30% in 3 type B outbreaks to 20% in a large type C outbreak to only 10% in a retrospective study of type A cases and outbreaks.[2–6] These survival rates are much lower than for hospitalized foals, which have an overall survival rate of 96%, and an 87.5% survival rate even when mechanical ventilation is required.[7,8] As anticipated, hospitalized people also have a very high survival rate (97–100%).[9]

Our hypothesis was that adult horses treated in a referral hospital would have improved survival compared to that for outbreak situations. However, we believed that survival would remain lower than what has been reported for foals and people because of the difficulty in providing adequate supportive care and mechanical ventilation to recumbent adult horses. In addition to establishing the survival rate for hospitalized adult horses, our aims were to identify predictors of nonsurvival and to provide descriptive information for hospitalized horses with botulism.

Abbreviations:

AST	aspartate aminotransferase
CI	confidence interval
CK	creatine kinase
GGT	gamma-glutamyl transpeptidase
IQR	interquartile range
OR	odds ratio
PCR	polymerase chain reaction
ROC	receiver operating characteristic

Materials and Methods

Study Population

This investigation was a retrospective study of horses greater than or equal to 6 months of age with a final diagnosis of botulism. The medical records system at the George D. Widener Large Animal Hospital at New Bolton Center, University of Pennsylvania School of Veterinary Medicine, was searched for all horses greater than or equal to 6 months of age with a final diagnosis of botulism between 1989 and 2013.

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Independent Variables

Data were retrieved from the records to evaluate independent variables for the development of two models. First, individual case histories as well as admission data were examined as potentially predictive of nonsurvival. Second, data from hospitalization, including specific information regarding *C. botulinum* testing, treatment, clinical course, and complications were examined as predictive of nonsurvival.

Historical data included signalment (weight, age, breed, and sex), estimated distance travelled to hospital, botulism vaccination status, access to large round bales of hay, presence of other botulism cases at the same farm, duration of clinical signs before presentation, presence of specific clinical signs (inappetence, colic, lethargy, weakness, tremors, dysphagia, abnormal gait, increased recumbency, total recumbency, weak tongue, weak tail or eyelids, drooling, nasal discharge, depression or behavior change, choke, and decreased gastrointestinal sounds or manure production), and reason for referral (botulism suspect, colic, choke or dysphagia, and neurologic or recumbent). Admission data included temperature, pulse, respiratory rate, and presence of specific clinical signs (dysphagia, weak tongue, weak eyelids, weak anal tone, muscle tremors, abnormal gait or stance, mydriatic pupils or slow pupillary light reflex, low head carriage, increased recumbency, weak tail tone, total recumbency, decreased gastrointestinal sounds, abnormal respiratory effort, drooling, foul mouth odor, depression or behavior change, dorsal displacement of the soft palate or voice change, and nasal discharge). Hematologic variables recorded included PCV, total protein, fibrinogen, white blood cell count, neutrophil count, lymphocyte count, creatinine, creatine kinase, aspartate aminotransferase, gamma-glutamyl transpeptidase, and total bilirubin. If blood gas analysis was performed, sample type (arterial or venous), pH, pCO_2, pO_2, and lactate concentration were recorded.

Testing information included test type (mouse bioassay, polymerase chain reaction (PCR) assay, or both), sample type (manure, gastrointestinal contents, serum, feed, wound exudate), and test result (positive/negative for toxin, spores, or both; *C. botulinum* serotype). Treatment information included botulinum antitoxin administration and timing (categorized as <6 hours after admission or >6 hours after admission), use of enteral or parenteral nutrition, antimicrobial drug use (categorized as β-lactam only, β-lactam + other drug, other drug, and metronidazole), anti-inflammatory or analgesic drug use, intranasal oxygen insufflation, intravenous fluid administration, other medications utilized (ophthalmic ointment, dermal ointment, sedatives), and physical therapy performed (passive range of motion exercises, turning, or sling use). Clinical course included development of total recumbency (inability to stand without assistance) at any point, number of days until ability to stand was regained, number of days until ability to eat was regained, and development of complications. Complications recorded included pneumonia, skin wounds or decubital ulcers, muscle abscesses, thrombophlebitis, abortion, colic, salmonellosis, corneal ulcers, facial paralysis, cellulitis, colitis or diarrhea, hyperlipemia, fever of unknown origin, or spontaneous death.

The primary outcome was survival. Duration of hospitalization was also recorded. For horses that were euthanized, reason for euthanasia was recorded when known (poor prognosis, development of complications, or financial limitations). Necropsy findings were recorded if available.

Statistical Analysis

Descriptive statistics and additional analyses were performed using a commercially available statistical software package.[a] Data for continuous variables were evaluated for normality using a Shapiro–Wilk test. Results for survivors and nonsurvivors were compared using the Wilcoxon rank-sum (equality of populations) test for nonnormally distributed continuous variables and an unpaired Student's *t*-test for normally distributed continuous variables. Fisher's exact test or chi-squared test was used for categorical data. All of these tests identified variables that were significantly different ($P < .05$) between survivors and nonsurvivors. For these significantly different variables, logistic regression was used to quantify the magnitude of the difference. When appropriate, differences between proportions were examined using two-sample tests of proportion.

A preliminary univariable screen of all predictor variables was performed to determine the association between variables and survival status, and to select those that could be included in multivariable analysis. Odds ratios were used to quantify associations. All exposures associated with survival in the univariable analysis at $P \leq .20$ were considered for inclusion in the multivariable analyses. Two multivariable models were created, one including historical and admission variables and one including hospitalization variables.

Performance of selected variables in either model was evaluated by purposeful backwards model selection. Model performance was evaluated using both Pearson's chi-squared and the Hosmer–Lemeshow test as summary statistics of goodness of fit ("calibration").[10,11] The area under the receiver operating characteristic (ROC) curve was determined as a summary statistic of discriminatory ability. Regression diagnostics were performed according to the methods of Hosmer and Lemeshow.[10] Covariate patterns and residuals were examined to identify and exclude (if indicated) patterns that fit poorly or had excessive influence on the values of the estimated parameters.

Results

Population Demographics and Descriptive Statistics

Ninety-two horses with a clinical diagnosis of botulism were identified. Not all examined data were available for every horse. Median age of the study population was 5 years (range, 0.5–25 years; interquartile range [IQR], 2–10 years), with 42 mares, 13 intact males, and 37 geldings. Represented breeds included 44 Thoroughbreds, 10 Standardbreds, 10 Quarter Horses, 8 miniature horses or ponies, 8 Arabians, 4 Warmbloods, 4 draft horses, 3 other light breeds, and 1 unknown. Median weight was 420 kg (range, 106–591 kg; IQR, 321–488 kg).

Access to large bales of hay was only recorded for 10 horses; 4/10 (40%) had access. The majority of horses (81/92; 88%) were isolated cases; the other 11 horses came from farms with at least one additional case. Vaccination status was recorded for 51 horses; 37 (73%) had never been vaccinated, 12 (24%) were incompletely vaccinated, and 2 (4%) were fully vaccinated.

Signs before admission included dysphagia (defined as difficulty swallowing as observed by the referring veterinarian or owner, noted in 54/92; 59%), weakness (35/92; 38%), increased recumbency (31/92; 34%), inappetence (23/92; 25%), abnormal gait (22/92; 24%), weak tongue (22/92; 24%), tremors (19/92; 21%), colic (17/92; 18%), lethargy (17/92; 18%), weak tail or eyelids (13/92; 14%), total recumbency (11/92; 12%), depression or behavior change (11/92; 12%), drooling (9/92; 10%), decreased gastrointestinal sounds or

manure production (9/92; 10%), nasal discharge (6/92; 7%), and choke (3/92; 3%). Median duration of signs was 1 day (range 0–9 days; IQR 0–2 days). Horses traveled a median of 50 miles to the hospital (range, 6–230 miles; IQR, 28–94 miles). The primary reason for admission was botulism suspect (52/92; 57%), though horses were also referred as colic cases (18/92; 20%), neurologic/down animals (10/92; 11%), or for choke or dysphagia (3/92; 3%).

Initial physical examination revealed a median temperature of 100.8°F (38.2°C) (range 94.5–104.0°F [34.7–40°C]; IQR 99.8–101.6°F [37.7–38.7°C]), a median heart rate of 60 beats/minute (range 28–120 beats/minute; IQR 44–70 beats/minute), and a median respiratory rate of 24 breaths/minute (range 8–66 breaths/minute; IQR 18–32 breaths/minute). The most common clinical signs noted by the admitting clinicians included weak tongue (85/92; 92%), weak eyelids (72/92; 78%), weak tail (72/92; 78%), dysphagia (69/92; 75%), tremors (45/92; 49%), decreased gastrointestinal sounds (41/92; 45%), abnormal gait (35/92; 38%), weak anal tone (30/92; 33%), abnormal pupils (26/92; 28%), increased recumbency (23/92; 25%), depression or behavior change (22/92; 24%), total recumbency (21/92; 23%), abnormal respiratory effort (16/92; 17%), drooling (12/92; 13%), nasal discharge (10/92; 11%), dorsal displacement of the soft palate (recorded for 8/92 (9%) cases but seen in 8/8 (100%) horses for which endoscopy was performed), and low head carriage (5/92; 5%). Initial hematologic and serum biochemical parameters of interest are presented in Table 1.

Table 1. Initial hematologic and serum biochemical results.

Test	Median	IQR	Range	Reference Range
PCV (%)	40	36–47	24–55	31.1–50.0
Total protein (g/dL)	7.2	6.8–7.8	5.2–9.7	5.8–7.5
White blood cell count (K/μL)	9.80	8.49–11.70	4.80–21.87	4.90–10.30
Neutrophil count (K/μL)	7.75	6.59–9.33	3.80–20.56	2.20–8.10
Lymphocyte count (K/μL)	1.66	1.31–2.21	0.40–4.12	1.70–5.80
Fibrinogen (mg/dL)	380	272–431	98–1,266	150–375
pH (venous)	7.41	7.38–7.43	7.25–7.47	7.35–7.45
pCO_2 (venous)	46.5	43.8–49.7	35.4–58.7	40–50
Lactate (mmol/L)	1.9	1.2–3.8	0.2–16.0	0–1.3
Creatinine (mg/dL)	1.6	1.4–2.0	1.0–5.8	0.6–1.8
CK (U/L)	475	241–864	87–9,631	90–270
AST (U/L)	508	374–688	223–2,380	205–555
GGT (U/L)	35	28–56	17–78	12–45
Total bilirubin (mg/dL)	3.8	3.1–4.8	2.3–6.3	0.1–1.9

IQR, interquartile range; CK, creatine kinase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase.

The majority of horses (83/92; 90%) had samples submitted for *C. botulinum* testing. Samples included manure (55/83; 66%), gastrointestinal contents (32/83; 39%), serum (7/83; 8%), feed (5/83; 6%), and wound exudate (6/83; 7%). Some horses had more than one type of sample submitted. All samples were analyzed via mouse bioassay; five samples also were analyzed via PCR. Positive results were obtained for 18/83 horses (22%) using mouse bioassay; 2/18 (11%) were positive only for toxin, 13/18 (72%) were positive for spores, and 3/18 (17%) were positive both for toxin and spores. PCR yielded positive results for 3/5 samples. The PCR positive samples were also positive on the bioassay, 2 for spores only and 1 for both toxin and spores.

Treatment information was available for 91 horses, although information about all treatments was not available for all patients. The two most common treatments were fluids administered IV (72/91; 79%) and pentavalent (A-E) botulinum antitoxin[b] (71/91; 78%). Timing of antitoxin administration was recorded for 66/71 horses; of these, 56/66 (85%) received antitoxin within 6 hours of admission. Other treatments included enteral nutrition (55/79; 70%), antimicrobial drugs (59/91; 65%), ophthalmic ointment (46/87; 53%), anti-inflammatory or analgesic medications (40/89; 45%), intranasal oxygen insufflation (24/86; 28%), parenteral nutrition (16/74; 22%), and dermal ointment (14/87; 16%). Physical therapy was instituted for 37/87 (43%) horses; therapy included frequent recumbency changes (36/37; 97%), assisting to stand with a sling (14/37; 38%), and passive range of motion exercises (2/37; 5%).

During the course of hospitalization, 49/92 (53%) horses became totally recumbent (unable to stand without assistance). Eleven recumbent horses regained the ability to stand, with a median time of 13 days (range 9–31 days; IQR 10–16 days). Nine of these 11 horses survived. Thirty-nine horses with complete dysphagia regained the ability to eat, with a median time of 8 days (range 2–16 days; IQR 6–11 days). Median duration of hospitalization for survivors was 14 days (range 2–51 days; IQR 9–20 days) and for nonsurvivors was 1 day (range 0–23 days; IQR 1–2 days).

Complications were common in hospitalized horses, developing in 56/90 (62%) horses for which information was available. The most common complications included decubital ulcers (30/90; 33%), pneumonia (22/90; 24%), corneal ulcers (17/90; 19%), and colic (9/90; 10%). Additional complications included cellulitis (8/90; 9%), salmonellosis (6/90; 7%), colitis or diarrhea (6/90; 7%), hyperlipemia or hyperlipidemia (6/90; 7%), thrombophlebitis (6/90; 7%), muscle abscesses (4/90; 4%), fever of unknown origin (4/90; 4%), facial paralysis (3/90; 3%), and abortion (1/90; 1%).

The overall survival rate was 48% (44/92 horses). However, survival among horses that arrived standing was significantly higher at 67% (40/60, $P = .011$), and even higher at 95% (35/37, $P < .001$) for horses that remained able to stand throughout hospitalization. The fact that there was also a significant difference in

survival between the horses that arrived standing and those that remained standing throughout hospitalization ($P < .001$) is indicative of a negative impact of recumbency on survival. Of the 48 nonsurvivors, 12 (25%) died spontaneously, and 36 (75%) were euthanized. Six horses were euthanized primarily for financial reasons, 1 was euthanized because of complications that arose during hospitalization, and the remaining 29 were euthanized based on poor prognosis. Horses euthanized for prognostic reasons invariably were totally recumbent.

Univariable Analysis

Results of the univariable screen pertaining to model 1 (historical and admission data) and model 2 (hospitalization data) are presented in Tables 2 and 3, respectively. Only variables considered for inclusion in the final models are listed. A number of interaction terms were examined, but none were found to be significant. The proportion of nonsurvivors unable to stand, both historically and at admission, was greater than that observed in the survivors (18.8% and 39.6% versus 4.6% and 4.6%, respectively, see Table 2). Likewise, becoming totally recumbent at any point during hospitalization was associated with poor outcome in horses with botulism (OR = 0.05; 95% CI, 0.01–0.20, $P < .001$, see Table 3). The median temperature of nonsurvivors (100.2°F [37.9°C]) was lower than that of survivors (101.2°F [38.4°C]), with $P = .001$. The proportion of nonsurvivors for which dysphagia was observed at admission (62.5%) was smaller than that of survivors (88.6%), with $P = .004$. The proportion of nonsurvivors with abnormal respiratory effort (29.2%) was larger than that of survivors (4.6%), with $P = .002$. The proportion of nonsurvivors receiving antitoxin (61.7%) was smaller than that of survivors (95.5%), with $P < .001$.

Multivariable Analysis

A large number of predictor variables had a $P < .20$ in the univariable analysis, but it was very apparent that combinations of quite a number of them were highly correlated. All variables eligible for consideration in the multivariable analysis were carefully examined for colinearity and multi-colinearity. Where correlations were detected the clinical importance of each variable was carefully assessed to identify those most plausibly linked to survival, rather than those that were surrogates for other variables eg, recumbency. Only the most clinically plausible variables were included in the final model building processes.

Model 1—Data available at admission: For information available at admission, when all other variables were controlled, the variables most strongly associated with outcome were rectal temperature, dysphagia, and abnormal respiratory effort (Table 4). Every degree Fahrenheit increase in rectal temperature increased the odds of survival by 94% (OR, 1.94; CI, 1.19–3.17; $P = .008$). Observation of dysphagia during initial clinical examination also increased the odds of survival by more than 300% (OR, 4.04; CI, 1.01–16.17; $P = .049$). Conversely, observation of abnormal respiratory effort during initial clinical examination decreased the odds of survival by 93% (OR, 0.07; CI, 0.01–0.65; $P = .019$). Regression diagnostics demonstrated that the model appropriately fit the data (Homer–Lemeshow $\chi^2 = 3.89$; $P = .867$). The area under the ROC curve was 0.801, suggesting that the model had "good" discriminating characteristics.[10,11] No covariate patterns were excluded.

Model 2—Data from hospitalization: Considering clinical findings during hospitalization, when all other variables were controlled, the variables most strongly associated with outcome were treatment with antitoxin and inability to stand (Table 4). Treatment with antitoxin greatly increased the odds of survival (OR, 121.30; CI, 9.94–1,480.65; $P < .001$), whereas inability to stand decreased the odds of survival by 99% (OR, 0.01; CI, 0.00–0.08; $P < .001$). Regression diagnostics demonstrated that the model appropriately fit the data ($\chi^2 = 0.03$; $P = .861$). The area under the ROC curve was 0.895, suggesting that the model had "good" to "excellent" discriminating characteristics.[9,10] No covariate patterns were excluded.

Discussion

The overall survival rate (48%) for hospitalized horses in this study was higher than rates reported in outbreak situations (10–30%). However, it remains much lower than survival rates for foals and humans with botulism. Development of sustained recumbency at any point during hospitalization was the variable most strongly associated with nonsurvival, and treatment with antitoxin was the variable most strongly associated with survival. Examination of the outcome for hospitalized horses yields potentially useful information. If the 6 horses that were euthanized for financial reasons during the early stages of treatment are removed from consideration, 86 treated horses are left. Of these, 37 horses never lost the ability to rise, and 35/37 (95%) survived. Forty-nine horses did lose the ability to rise, and only 9/49 (18%) survived. Therefore, clinicians would be justified in giving a poor prognosis (20% or less) to horses with botulism that lose the ability to rise, regardless of aggressive hospital treatment. Conversely, horses that retain the ability to stand throughout hospitalization have an excellent prognosis, with a 95% survival rate.

However, it is important to recognize that, although botulism is usually a rapidly progressive disease, horses might retain the ability to stand during the first few days of treatment and then become totally recumbent. This deterioration is seen despite treatment with botulinum antitoxin because antitoxin only binds circulating toxin and does not remove toxin already bound to receptors at the motor end plate. Of the 49 treated horses that lost the ability to stand, 31/49 (63%) were totally recumbent within 24 hours of hospitalization (data not shown). An additional 10/49 (20%) lost the ability to stand on the second day of hospitalization, with 5/49 (10%), 1/49 (2%), and 2/49 (4%) losing the ability to stand on the third, fourth, and fifth days of

Table 2. Univariable logistic regression analysis of historical and admission variables and survival in adult horses diagnosed with botulism. Only variables with $P < .20$ that were considered for inclusion in the final model are listed.

Variable	Nonsurvivors (n = 48) n (%) *Median (IQR)*	Survivors (n = 44) n (%) *Median (IQR)*	Crude OR	95% CI	*P*-Value
Signalment					
Weight (kg)	*377 (295–436)*	*448 (394–495)*	1.01	1.00–1.01	.120
Age (years)	*7 (3–12)*	*3.5 (2–8)*	0.93	0.86–1.01	.089
Breed					
Thoroughbred	20 (42.6)	24 (54.6)	Reference		NA
Standardbred	4 (8.5)	6 (13.6)	1.25	0.31–5.06	.754
Warmblood	3 (6.4)	1 (2.3)	0.28	0.03–2.88	.283
Quarter Horse	8 (17.0)	2 (4.6)	0.21	0.04–1.10	.064
Arabian	1 (2.1)	2 (4.6)	1.67	0.14–19.76	.686
Miniature/pony	6 (12.8)	2 (4.6)	0.28	0.05–1.53	.141
Other light breed	1 (2.1)	3 (6.8)	2.50	0.24–25.95	.443
Draft	4 (8.5)	4 (9.1)	0.83	0.19–3.76	.813
Historical signs					
Colic					
No	36 (75)	39 (88.6)	Reference		NA
Yes	12 (25)	5 (11.4)	0.39	0.12–1.23	.094
Sustained recumbency					
No	39 (81.3)	42 (95.5)	Reference		NA
Yes	9 (18.8)	2 (4.6)	0.21	0.04–1.07	.037
Weak tail/eyelid					
No	39 (81.3)	40 (90.9)	Reference		NA
Yes	9 (18.8)	4 (9.1)	0.43	0.12–1.55	.186
Nasal discharge					
No	43 (89.6)	43 (97.7)	Reference		NA
Yes	5 (10.4)	1 (2.3)	0.20	0.02–1.86	.116
Reason for admit					
Botulism suspect					
No	23 (48.9)	15 (34.9)	Reference		NA
Yes	24 (51.1)	28 (65.1)	1.79	0.76–4.24	.180
Colic					
No	35 (74.5)	37 (86.1)	Reference		NA
Yes	12 (25.5)	6 (14.0)	0.47	0.16–1.42	.173
Clinical signs					
Temperature	*100.2 (99.1–101.4)*	*101.2 (100.8–101.8)*	2.10	1.36–3.23	.001
Respiratory rate	*24 (20–32)*	*24 (16–28)*	0.97	0.93–1.01	.154
Dysphagia					
No	18 (37.5)	5 (11.4)	Reference		NA
Yes	30 (62.5)	39 (88.6)	4.68	1.47–14.92	.004
Weak tongue					
No	1 (2.1)	6 (13.6)	Reference		NA
Yes	47 (97.9)	38 (86.4)	0.14	0.02–1.25	.038
Weak eyelids					
No	6 (12.5)	14 (31.8)	Reference		NA
Yes	42 (87.5)	30 (68.2)	0.31	0.10–0.92	.026
Weak anal tone					
No	29 (60.4)	33 (75)	Reference		NA
Yes	19 (39.6)	11 (25)	0.51	0.21–1.27	.138
Abnormal pupils					
No	30 (62.5)	36 (81.8)	Reference		NA
Yes	18 (37.5)	8 (18.2)	0.37	0.14–1.00	.041
Increased recumbency					
No	33 (68.8)	36 (81.8)	Reference		NA
Yes	15 (31.3)	8 (18.2)	0.49	0.18–1.32	.150
Weak tail					
No	7 (14.6)	13 (29.6)	Reference		NA
Yes	41 (85.4)	31 (70.5)	0.41	0.14–1.17	.084

Table 2. Continued

Variable	Nonsurvivors (n = 48) n (%) *Median (IQR)*	Survivors (n = 44) n (%) *Median (IQR)*	Crude OR	95% CI	*P*-Value
Total recumbency					
No	29 (60.4)	42 (95.5)	Reference		NA
Yes	19 (39.6)	2 (4.6)	0.07	0.01–0.40	<.001
Decreased GI sounds					
No	22 (45.8)	29 (65.9)	Reference		NA
Yes	26 (54.2)	15 (34.1)	0.44	0.18–1.04	.054
Abnormal respiratory effort					
No	34 (70.8)	42 (95.5)	Reference		NA
Yes	14 (29.2)	2 (4.6)	0.12	0.02–0.60	.002
Hematology					
Hematocrit (%)	*43 (39–46)*	*38 (35–47)*	0.95	0.89–1.02	.151
Total protein (g/dL)	*7.3 (7.0–7.8)*	*7.0 (6.6–7.6)*	0.69	0.40–1.19	.184
WBC count (K/μL)	*10.40 (8.86–12.25)*	*9.66 (8.16–10.56)*	1.00	1.00–1.00	.070
Neutrophil count (K/μL)	*8.74 (6.75–9.91)*	*7.10 (6.31–8.60)*	1.00	1.00–1.00	.044
pH	*7.41 (7.36–7.43)*	*7.42 (7.41–7.43)*	4043459	0.17–9.78e+13	.079
pCO_2 (mmHg)	*47.7 (45.9–51.5)*	*45.1 (43–46.8)*	0.86	0.74–1.00	.043
CK (U/L)	*600 (327–1,616)*	*300 (174–696)*	1.00	0.99–1.00	.043
AST (U/L)	*476 (367–665)*	*543 (389–734)*	1.00	0.99–1.00	.136

IQR, interquartile range; OR, odds ratio; CI, confidence interval; CK, creatine kinase; AST, aspartate aminotransferase.

hospitalization, respectively. Clinicians should warn owners of the small number of horses with a delayed onset of total recumbency, meaning that the most accurate prognosis can only be provided after the horse has been hospitalized for 3–5 days. If only the equine's admission status is considered, this study showed that 40/60 (67%) of horses that arrive standing survived, compared to 4/31 (13%) of horses that arrived recumbent. These numbers suggest that early intervention is critical, and that veterinarians should aim to treat or refer cases before development of recumbency.

Other than recumbency and treatment with antitoxin, variables that predicted survival included several initial clinical examination findings. Rectal temperature was lower in nonsurvivors, likely representing more severe neuromuscular weakness (decreased anal and rectal tone) as well as potentially cardiovascular compromise. Dysphagia was observed in a larger proportion of survivors than nonsurvivors. One potential explanation for this finding is that horses with obvious dysphagia were more quickly identified as botulism cases and treated appropriately. Another possibility is that horses that showed obvious dysphagia were less severely affected than horses that did not; dysphagia is considered an early sign of botulism and is often observed in ambulatory horses. Horses more severely affected with botulism are often totally recumbent and increasingly distressed, potentially losing interest in food such that dysphagia becomes less evident. Finally, abnormal respiratory effort was a strong predictor of nonsurvival, consistent with a severe degree of diffuse neuromuscular weakness and end-stage disease.

Although all horses came from a region where botulism is relatively common, only 57% were referred to the hospital as botulism suspects. Twenty percent were referred as colic cases, and 11% as neurologic/down animals. Several were referred with a presumptive diagnosis of esophageal obstruction. Surprisingly, only 1 of the botulism suspects was treated with antitoxin before referral. This suspect was a herdmate of a pony already hospitalized for botulism; antitoxin was provided by New Bolton Center so that the herdmate could be treated before shipping on the same day. Early identification and treatment of horses with botulism before the onset of recumbency are the most important contributing factors to survival. Therefore, clinicians working in regions where botulism is endemic should maintain a high index of suspicion for the disease, particularly when examining horses for colic. In addition, with the current availability of reasonably priced, commercially available antitoxin, clinicians are encouraged to stock at least 1 unit of antitoxin so that treatment can be provided before referral for more intensive care.

Clinical diagnosis of botulism remains the standard of care because of problems inherent in identifying botulinum toxin in equine samples.[1,6,12,13] Approximately 30% of adult horses with botulism have positive test results using mouse bioassay.[1] Only 22% of horses with a final diagnosis of botulism had positive laboratory results using mouse bioassay in this study. Test results were not associated with survival, suggesting that obtaining a positive result might be related to timing of sample collection rather than indicative of a higher toxin load. PCR testing yielded a 60% positive rate but was only used in 5 cases, and all 3 PCR positive samples were also positive using mouse bioassay. This PCR assay was only recently validated and has been reported to have a higher sensitivity than the mouse bioassay.[12,13] However, more clinically diagnosed cases need to be evaluated with PCR testing before its true sensitivity in this patient population is known.

Table 3. Univariable logistic regression analysis of hospitalization variables and survival in adult horses diagnosed with botulism. Only variables with $P < .20$ that were considered for inclusion in the final model are listed.

Variable	Nonsurvivors (n = 48) n (%)	Survivors (n = 44) n (%)	Crude OR	95% CI	*P*-Value
Treatment					
Antitoxin					
No	18 (38.3)	2 (4.6)	Reference		NA
Yes	29 (61.7)	42 (95.5)	13.03	2.40–70.69	<.001
<6 hours					
No	21 (47.7)	9 (21.4)	Reference		NA
Yes	23 (52.3)	33 (78.6)	3.35	1.25–9.00	.011
Enteral nutrition					
No	21 (56.8)	3 (7.1)	Reference		NA
Yes	16 (43.2)	39 (92.9)	17.06	3.43–84.77	<.001
Antimicrobial class					
B-lactam alone	18 (62.1)	5 (17.9)	Reference		NA
B-lactam + other	5 (17.2)	16 (57.1)	11.52	2.81–47.22	.001
Other	4 (13.8)	3 (10.7)	2.70	0.45–16.26	.278
Metronidazole	2 (6.9)	4 (14.3)	7.20	1.01–51.39	.049
Oxygen					
No	28 (62.2)	34 (82.9)	Reference		NA
Yes	17 (37.8)	7 (17.1)	0.34	0.12–0.97	.034
IV fluids					
No	5 (10.4)	14 (32.6)	Reference		NA
Yes	43 (89.6)	29 (67.4)	0.24	0.07–0.78	.010
Other meds					
No	9 (19.6)	16 (38.1)	Reference		NA
Yes	37 (80.4)	26 (61.9)	0.40	0.15–1.06	.056
Ophthalmic oint.					
No	16 (35.6)	25 (59.5)	Reference		NA
Yes	29 (64.4)	17 (40.5)	0.38	0.15–0.92	.026
Skin ointment					
No	41 (91.1)	32 (76.2)	Reference		NA
Yes	4 (8.9)	10 (23.8)	3.20	0.89–11.55	.060
Physical therapy					
No	18 (40)	32 (76.2)	Reference		NA
Yes	27 (60)	10 (23.8)	0.21	0.08–0.57	.001
Turning					
No	19 (42.2)	32 (76.2)	Reference		NA
Yes	26 (57.8)	10 (23.8)	0.23	0.09–0.62	.001
Sustained recumbency					
No	8 (16.7)	35 (79.6)	Reference		NA
Yes	40 (83.3)	9 (20.5)	0.05	0.01–0.20	<.001
Complications					
Cellulitis					
No	45 (97.8)	37 (84.1)	Reference		NA
Yes	1 (2.2)	7 (15.9)	8.51	0.93–78.19	.023

OR, odds ratio; CI, confidence interval.

Although determination of toxin type requires laboratory testing, it is likely that all horses in this study had type B botulism, which is endemic in mid-Atlantic states.[6] Indeed, for the 22% of horses with positive test results, all were confirmed to have type B botulism. Survival rate might vary with *C. botulinum* serotype; people with type A botulism tend to have more severe disease than those with type B, with faster onset of paralysis and increased likelihood of requiring mechanical ventilation, and a higher case fatality rate.[14] However, since type B cases are most common and are estimated to account for over 85% of equine cases diagnosed in the United States,[1] results of this study provide a good estimation of expected prognosis.

Complications occurred in the majority of horses hospitalized for botulism but did not influence survival. Decubital ulcers, the most frequent complication (33% of cases), can be directly attributed to excessive recumbency, as can several other complications (corneal ulcers, cellulitis, muscle abscessation, and facial paralysis). Pneumonia, the second-most frequent complication (24% of cases), can be caused by aspiration secondary to dysphagia and potentially worsened by compression atelectasis secondary to recumbency. Gastrointestinal complications were also relatively common (colic in

Table 4. Variables retained in the final multivariable models. Model 1 includes associations between criteria available on admission (signalment, history, clinical signs, and initial hematology) and survival in adult horses with botulism. Model 2 includes associations between criteria from hospitalization (treatment, clinical course, and complications) and survival in adult horses with botulism.

Variable	Nonsurvivors (n = 48) n (%) *Median (IQR)*	Survivors (n = 44) n (%) *Median (IQR)*	Adjusted OR	95% CI	*P*-Value
Model 1					
Temperature	*100.2 (99.1–101.4)*	*101.2 (100.8–101.8)*	1.94	1.19–3.17	.008
Dysphagia					
No	18 (37.5)	5 (11.4)	Reference		
Yes	30 (62.5)	39 (88.6)	4.04	1.01–16.17	.049
Abnormal respiratory effort					
No	34 (70.8)	42 (95.5)	Reference		
Yes	14 (29.2)	2 (4.6)	0.07	0.01–0.65	.019
Model 2					
Antitoxin treatment					
No	18 (38.3)	2 (4.6)	Reference		
Yes	29 (61.7)	42 (95.5)	121.30	9.94–1,480.65	<.001
Sustained recumbency					
No	8 (16.7)	35 (79.6)	Reference		
Yes	40 (83.3)	9 (20.5)	0.01	0.00–0.08	<.001

IQR, interquartile range; OR, odds ratio; CI, confidence interval.

10% of cases, salmonellosis in 7%, and colitis in 7%). Although the overall rate of salmonellosis was low in botulism cases, it is higher than what is seen in our normal hospital population (excluding high-risk animals, ie, equine colics, bovines, and colitis cases), which has a shedding rate of 1.2% (H. Aceto, Personal communication, and higher than what is reported nationally.[c] The increased rate of salmonellosis in horses with botulism could indicate that these horses were carriers and had enough gastrointestinal upset to develop clinical disease. However, our clinical impression, supported by culture and biotype data (not shown), is that these horses developed hospital-associated infections (HAI). Horses with botulism are likely at higher risk for HAI because of increased time spent recumbent, with their mouths and often their tongues in direct contact with the floor, as well as frequent enteral feeding via nasogastric intubation (usually 2–4 times/day for 1–2 weeks), with potential introduction of pathogenic bacteria each time a tube is passed. Therefore, careful attention to biosecurity and barrier precautions should be considered for horses with botulism to prevent HAI.

Only one USDA-approved vaccine[d] against equine botulism is available in the United States (BotVax B[d]). This product is a killed (toxoid) vaccine directed against *C. botulinum* type B. For horses for which vaccination history was available, 49/51 (96%) were unvaccinated or not fully vaccinated (defined as an initial series of 3 doses administered at 4 week intervals, with subsequent yearly boosters). Only 2 horses with botulism were reportedly fully vaccinated. Both of these horses had mild clinical signs, including dysphagia but not diffuse weakness or excessive recumbency, and both survived. Interestingly, these are the first 2 horses reported in the literature to develop botulism despite an adequate vaccination history. One of the horses was confirmed positive for type B spores on mouse bioassay, representing a confirmed vaccine failure. The other horse was negative on mouse bioassay and thus toxin type was undetermined. Although there is a small possibility that this horse was affected by a different toxin type, and therefore does not represent a true vaccine failure, the horse almost certainly had type B botulism as it came from Pennsylvania. In this study, all cases with positive test results were confirmed type B, and to the authors' knowledge there have not been confirmed type A or C botulism cases in horses from Pennsylvania. Importantly, there is no cross-protection between serotypes, so the toxoid vaccine would not be expected to prevent types A or C botulism.[1]

In summary, owners of horses diagnosed with botulism can generally anticipate a 2-week hospitalization period, although the range encompassed 2–51 day stays. Nonsurviving horses were generally euthanized quickly, with a median duration of hospitalization of 1 day. Maintaining the ability to stand is the most important predictor of survival, and an excellent prognosis for recovery is warranted for horses that do not become recumbent. Conversely, loss of the ability to stand confers a poor prognosis. Complications should be expected and anticipated, with precautions taken to minimize the adverse effects of excessive recumbency, aspiration, and gastrointestinal dysregulation.

Footnotes

[a] STATA IC version 13.1, StataCorp LP, College Station, TX

[b] Pentavalent (A-E) botulinum antitoxin was produced in-house by the Botulism Reference Laboratory using a hyperimmunized plasma donor horse

[c] USDA-APHIS, Center for Epidemiology and Animal Health. *Salmonella* and the U.S. horse population. 2001. Available at: http://www.aphis.usda.gov/animal_health/nahms/equine/down-

loads/equine98/Equine98_is_Salmonella.pdf. Accessed May 15, 2014

[d] BotVax B, *Clostridium botulinum* Type B Toxoid, Neogen Corporation, Lexington, KY

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Conflict of Interest Declaration: The authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: The authors declare no off-label use of antimicrobials.

References

1. Whitlock RH, McAdams S. Equine botulism. Clin Tech Equine Pract 2006;5:37–42.

2. Haagsma J, Haesebrouck F, Devriese L, et al. An outbreak of botulism type B in horses. Vet Rec 1990;127:206.

3. Ricketts SW, Greer TR, Glyn PJ, et al. Thirteen cases of botulism in horses fed big bale silage. Equine Vet J 1984;16:515–518.

4. Schoenbaum MA, Hall SM, Glock RD, et al. An outbreak of type C botulism in 12 horses and a mule. J Am Vet Med Assoc 2000;217:365–368.

5. Kinde H, Bettey RL, Ardans A, et al. Clostridium-botulinum type-C intoxication associated with consumption of processed alfalfa hay cubes in horses. J Am Vet Med Assoc 1991;199:742–746.

6. Johnson AL, McAdams SC, Whitlock RH. Type A botulism in horses in the United States: A review of the past ten years (1998–2008). J Vet Diagn Invest 2010;22:165–173.

7. Wilkins PA, Palmer JE. Botulism in foals less than 6 months of age: 30 cases (1989–2002). J Vet Intern Med 2003;17:702–707.

8. Wilkins PA, Palmer JE. Mechanical ventilation in foals with botulism: 9 cases (1989–2002). J Vet Intern Med 2003;17:708–712.

9. Centers for Disease Control and Prevention (CDC). Botulism Annual Summary, 2011. Atlanta, GA: US Department of Health and Human Services, CDC; 2013.

10. Hosmer DW, Lemeshow S. Applied Logistic Regression, 2nd ed. New York: J. Wiley and Sons Inc; 2000:91–186.

11. Dohoo I, Martin W, Stryhn H. Veterinary Epidemiologic Research, 2nd ed. Prince Edward Island: VER Inc; 2009:410–421.

12. Johnson AL, Sweeney RW, McAdams SC, et al. Quantitative real-time PCR for detection of the neurotoxin gene of *Clostridium botulinum* type B in equine and bovine samples. Vet J 2012;194:118–120.

13. Johnson AL, McAdams-Gallagher SC, Sweeney RW. Quantitative real-time PCR for detection of neurotoxin genes of Clostridium botulinum types A, B, and C in equine samples. Vet J 2014;199:157–161.

14. Horowitz BZ. Botulinum toxin. Crit Care Clin 2005;21:825–839.