

Workgroup Report by the Joint Task Force Involving American Academy of Allergy, Asthma & Immunology (AAAAI); Food Allergy, Anaphylaxis, Dermatology and Drug Allergy (FADDA) (Adverse Reactions to Foods Committee and Adverse Reactions to Drugs, Biologicals, and Latex Committee); and the Centers for Disease Control and Prevention Botulism Clinical Treatment Guidelines Workgroup—Allergic Reactions to Botulinum Antitoxin: A Systematic Review

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Background. Naturally occurring botulism is rare, but a large number of cases could result from unintentional or intentional contamination of a commercial food. Despeciated, equine-derived, heptavalent botulinum antitoxin (HBAT) is licensed in the United States. Timely treatment reduces morbidity and mortality, but concerns that botulinum antitoxin can induce anaphylaxis exist. We sought to quantify the allergy risk of botulinum antitoxin treatment and the usefulness of skin testing to assess this risk.

Methods. We conducted a systematic review of (1) allergic reactions to botulinum antitoxin and (2) the predictive value of skin testing (ST) before botulinum antitoxin administration. We searched 5 scientific literature databases, reviewed articles' references, and obtained data from the HBAT manufacturer and from the Centers for Disease Control and Prevention. Anaphylaxis incidence was determined for HBAT and previously employed botulinum antitoxins. We calculated the positive predictive value (PPV) and negative predictive value (NPV) of ST for anaphylaxis related to HBAT and other botulinum antitoxins.

Results. Seven articles were included. Anaphylaxis incidence was 1.64% (5/305 patients) for HBAT and 1.16% (8/687 patients) for all other botulinum antitoxins (relative risk, 1.41 [95% confidence interval, .47–4.27]; $P = .5$). Observed values for both PPV and NPV for HBAT-ST (33 patients) were 100%. Observed PPVs and NPVs of ST for other botulinum antitoxins (302 patients) were 0–56% and 50%–100%, respectively. There were no reports of fatal anaphylaxis.

Conclusions. Considering the <2 % rate of anaphylaxis, fatal outcomes, modest predictive value of ST, resource requirements for ST, and the benefits of early treatment, data do not support delaying HBAT administration to perform ST in a mass botulinum toxin exposure. Anaphylactic reactions may occur among 1%–2% of botulinum antitoxin recipients and will require epinephrine and antihistamine treatment and, possibly, intensive care.

Keywords. botulism; botulinum antitoxin; equine antitoxin; allergy; anaphylaxis.

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Botulism is a rare, potentially fatal, paralytic disease caused by neurotoxins produced by *Clostridium botulinum* and, more rarely, by related clostridia [1, 2]. There are 6 phylogenetically distinct clostridia, which produce 7 serotypically and antigenically distinct botulinum toxin types, designated by the letters A through G [1, 3, 4]. These 7 botulinum toxin serotypes A–G were described between 1919 and 1970. Botulinum serotypes were first identified through serological methods, and the letters A–G signify the order in which these toxins were identified

[5]. Strains were identified as antigenically distinct if neutralizing antibodies raised by inoculation of an animal with one neurotoxin would not cross-neutralize the neurotoxin formed by another strain [5]. Thus, serum antibody raised for antitoxin against one serotype does not cross-react with other serotypes [5]. Recently, reports of new toxin types have been published, including a novel toxin subsequently shown to be hybrid type A/F fully neutralized by heptavalent botulinum antitoxin (HBAT) [6, 7], and a novel toxin identified and assembled from the published gene sequence of a *C. botulinum* isolate [8]. Because new botulinum toxins of clinical significance might be discovered, preparedness requires careful laboratory investigation of all suspected botulism cases and research and development of new countermeasures.

Botulism has 4 recognized clinical syndromes: foodborne botulism, wound botulism, infant botulism, and adult intestinal toxemia [1, 3]. Nonnaturally occurring forms of botulism can include iatrogenic and inhalation botulism. There are case reports of iatrogenic botulism due to botulinum toxin A, used therapeutically for neurologic disorders and cosmetic treatment [9]. A sixth, nonnatural form, is inhalation botulism, which can result from the aerosolization of toxin [3]. Botulinum causes paralysis by blocking acetylcholine transmission across the neuromuscular junction [1]. All forms of botulism produce the same distinct clinical syndrome of symmetrical cranial nerve palsies, followed by descending, symmetric, flaccid paralysis of voluntary muscles. This reaction can progress to respiratory compromise and death [1, 3, 10]. Botulinum toxin has been weaponized by various countries as an agent of biological warfare [11]. Mass-exposure events could be caused by natural or intentional contamination of commercialized foods or deliberate aerosolization [11].

Currently used therapy for botulism consists of intensive supportive care and treatment with equine-source antitoxin. Botulinum antitoxin consists of equine-derived immunoglobulin G (IgG) antibodies directed against the antigenically distinct botulinum neurotoxins. These antibodies neutralize circulating neurotoxin molecules by coating the molecules not yet bound to nerve endings and preventing attachment to neurons [2, 10]. Antitoxin does not reverse existing paralysis [11, 12]. Therefore, botulinum antitoxin must be administered as early as possible after symptom onset.

Despeciation and other processes used in producing modern equine antitoxin substantially, but not completely, decrease the risk of allergic reactions, including anaphylaxis. Skin testing (ST) of patients before the antitoxin is administered may provide some indication of the level of risk, but the person who does this testing must have specialized training, the process is labor intensive and time consuming (20–60 minutes), and the predictive value of the test results is uncertain. In a mass-exposure event, a substantial risk of anaphylaxis would create a large additional care burden, and ST would only be useful if it

were feasible and resulted in substantial reduction in the risk of anaphylaxis.

We conducted a systematic review of human reports involving hypersensitivity reactions and ST to horse dander or equine-derived botulinum antitoxin. Our goal was to compile evidence on which to base recommendations for pretreatment ST in the event of a large botulinum exposure. Specifically, we sought to (1) determine the risk (rate) of anaphylaxis and (2) assess the usefulness of ST. Reports of allergic reactions, anaphylaxis, and ST accuracy are included in this review.

METHODS

Data Sources and Search Strategy

We used systematic methods to identify published descriptions of allergic reactions to botulinum antitoxins. A search strategy was developed by subject-matter experts (SMEs) in the field of allergy and immunology at the Icahn School of Medicine at Mount Sinai, at Northwestern University's Feinberg School of Medicine, and by librarians at the Centers for Disease Control and Prevention (CDC). This review follows the methods outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [11, 13]. The search string terms (Table 1), selected in consultation with an expert CDC reference librarian and with SMEs, were run to maximally capture all published literature related to ST and adverse reactions to botulinum antitoxins. Five databases were searched from their inception to November 2015: Medline (1946–) Embase (1947–), Global Health (1910–), Scopus (1960–), and Cochrane Library (1800–). Studies that met a priori inclusion criterion included randomized controlled trials; case-control studies; cross-sectional, observational studies; and case series that involved hypersensitivity or ST to antitoxin. Consistent search terms were used among databases (Table 1). We identified additional studies through hand-searching of references and communication with SMEs. Data on HBAT were provided by Cangene Corporation regarding 2 clinical trials of 56 healthy adult subjects (2006–2009) and by CDC's Botulism Clinical Consult Service regarding 249 botulism patients treated with HBAT (2010–2013).

Guiding Questions for the Literature Review

The following questions guided our literature review: (1) What is the risk of anaphylaxis to HBAT? (2) What is the utility of ST for predicting anaphylaxis to HBAT? (3) How does HBAT allergenicity compare with those of previous formulations of botulinum antitoxin? (4) Do factors such as history of atopy, asthma, or previous exposure to equine products increase the risk of an allergic reaction to botulinum antitoxin?

Data Extraction and Analysis

An Excel data abstraction tool adapted from other systematic reviews for biothreats was used to extract the following: author, year of study, country where study was performed, antitoxin

Table 1. Systematic Review of Allergic Reactions in Persons Receiving Equine Botulinum Antitoxin Products: Results With Search-String Terms

Database	Strategy	Download	Run Date
Medline (Ovid) 1946–	Exp hypersensitivity/ OR anaphylaxis/ OR serum sickness/ OR exp Asthma/ OR exp Rhinitis, Allergic/ OR exp Urticaria/ OR (hypersensitiv* OR serum sickness OR asthma* OR allerg* OR hay fever OR anaphyla* OR wheezing OR (chest ADJ2 tight*) OR dyspnea OR ((shortness OR labor* OR difficulty OR stop* OR trouble) ADJ2 breath*) OR urticaria* OR rash OR hives OR pruritus OR itch* OR angioedema OR vomit* OR nausea OR abdominal pain* OR swelling OR palpitation* OR shock OR (adverse ADJ2 (event* OR reaction* OR effect*)))ti,ab,kf,hw. OR adverse effects.fs. AND exp antitoxins/ OR exp Immune sera/ OR exp Immunoglobulins/ OR (Antisera OR antiserum* OR anti-sera OR anti-se- rum* OR immune sera OR immune serum* OR antitoxin* OR anti-toxin* OR HBAT OR HBAT OR antivenin* OR anti- venin* OR antivenom* OR anti-venom* OR immune globulin OR immunoglobulin OR immune plasma).ti,ab,kf,hw. AND (horse OR equine OR despeciated).mp NOT (exp Animals/ not exp humans/)	405	27 Oct 2015
Embase (Ovid) 1947–	Exp hypersensitivity/ OR anaphylaxis/ OR serum sickness/ OR exp Asthma/ OR exp Allergic Rhinitis/ OR exp Urticaria/ OR angioneurotic edema/ OR (hypersensitiv* OR serum sickness OR asthma* OR allerg* OR hay fever OR anaphyla* OR wheezing OR (chest ADJ2 tight*) OR dyspnea OR ((shortness OR labor* OR difficulty OR stop* OR trouble) ADJ2 breath*) OR urticaria* OR rash OR hives OR pruritus OR itch* OR angioedema OR vomit* OR nausea OR abdominal pain* OR swelling OR palpitation* OR shock OR (adverse ADJ2 (event* OR reaction*)))ti,ab,kw,hw. AND exp antitoxin/ OR exp antiserum/ OR exp Immunoglobulin/ OR (Antisera OR antiserum* OR anti-sera OR anti-serum* OR immune sera OR immune serum* OR antitoxin* OR anti-toxin* OR HBAT OR HBAT OR antivenin* OR anti-venin* OR antivenom* OR anti-venom* OR immune globulin OR immunoglobulin OR immune plasma).ti,ab,kw,hw. AND (horse OR equine OR despeciated).mp NOT (exp Animals/ not exp humans/)	566	27 Oct 2015
Global Health (Ovid) 1910–	Exp hypersensitivity/ OR anaphylaxis/ OR exp Asthma/ OR exp allergic rhinitis/ OR exp Urticaria/ OR (hypersensitiv* OR serum sickness OR asthma* OR allerg* OR hay fever OR anaphyla* OR wheezing OR (chest ADJ2 tight*) OR dyspnea OR ((shortness OR labor* OR difficulty OR stop* OR trouble) ADJ2 breath*) OR urticaria* OR rash OR hives OR pru- ritus OR itch* OR angioedema OR vomit* OR nausea OR abdominal pain* OR swelling OR palpitation* OR shock OR (adverse ADJ2 (event* OR reaction* OR effect*)))ti,ab,hw. AND exp antitoxins/ OR exp Immune serum/ OR exp Immunoglobulins/ OR (Antisera OR antiserum* OR anti-sera OR anti-se- rum* OR immune sera OR immune serum* OR antitoxin* OR anti-toxin* OR HBAT OR HBAT OR antivenin* OR anti- venin* OR antivenom* OR anti-venom* OR immune globulin OR immunoglobulin OR immune plasma).ti,ab,hw. AND (horse OR equine OR despeciated).mp	337	27 Oct 2015
Scopus 1960–	((INDEXTERMS(hypersensitivity OR anaphylaxis OR Asthma OR "allergic rhinitis" OR Urticaria OR "serum sickness") OR TITLE-ABS-KEY(hypersensitiv* OR "serum sickness" OR asthma* OR allerg* OR "hay fever" OR anaphyla* OR wheezing OR (chest W/2 tight*) OR dyspnea OR (shortness W/2 breath*) OR (labor* W/2 breath*) OR (difficulty W/2 breath*) OR (stop* W/2 breath*) OR (trouble W/2 breath*) OR urticaria* OR rash OR hives OR pruritus OR itch* OR angioedema OR vomit* OR nausea OR "abdominal pain*" OR swelling OR palpitation* OR shock OR (adverse W/2 event*) OR (adverse W/2 reaction*) OR (adverse W/2 effect*))) AND (INDEXTERMS(antitoxin OR antiserum OR Immunoglobulin) OR TITLE-ABS-KEY(Antisera OR antiserum* OR anti-sera OR anti-serum* OR "immune sera" OR "immune serum*" OR antitoxin* OR anti-toxin* OR HBAT OR HBAT OR antivenin* OR anti-venin* OR antivenom* OR anti-venom* OR "immune globulin" OR immunoglobulin OR "immune plasma")) AND (INDEXTERMS(horse OR equine OR despeciated) OR TITLE-ABS-KEY(horse OR equine OR despeciated)) AND Human OR Humans	537	27 Oct 2015
Cochrane Library 1800–	[mh hypersensitivity] OR [mh anaphylaxis] OR [mh "serum sickness"] OR [mh Asthma] OR [mh "Rhinitis, Allergic"] OR [mh Urticaria] OR (hypersensitiv* OR "serum sickness" OR asthma* OR allerg* OR "hay fever" OR anaphyla* OR wheezing OR (chest NEAR/2 tight*) OR dyspnea OR ((shortness OR labor* OR difficulty OR stop* OR trouble) NEAR/2 breath*) OR urticaria* OR rash OR hives OR pruritus OR itch* OR angioedema OR vomit* OR nausea OR "abdominal pain*" OR swelling OR palpitation* OR shock OR (adverse NEAR/2 (event* OR reaction* OR effect*)))ti,ab AND [mh antitoxins] OR [mh "Immune sera"] OR [mh Immunoglobulins] OR (Antisera OR antiserum* OR anti-sera OR anti-se- rum* OR "immune sera" OR "immune serum*" OR antitoxin* OR anti-toxin* OR HBAT OR HBAT OR antivenin* OR anti-venin* OR antivenom* OR anti-venom* OR "immune globulin" OR immunoglobulin OR "immune plasma"):ti,ab AND (horse OR equine OR despeciated)	38	27 Oct 2015

Asterisk (*) indicates wildcard (captures the preceding written term and any combination of characters following).

Abbreviations: HBAT, heptavalent botulinum antitoxin.

serotype, study design, demographics of study participants (including age and gender), atopic history, eligibility criteria, equine antitoxin product, despeciation information, number of persons treated, dose of antitoxin administered, route of administration, number and description of hypersensitivity reactions, ST method and test results, pretreatments, and desensitization information. Cangene data from 2 clinical trials and data on botulism patients treated with HBAT provided by CDC from government stockpiles were used to evaluate skin test, allergic reaction, and anaphylaxis data associated with administration of HBAT. To assess ST, we calculated the positive predictive value (PPV; the probability that subjects with a positive screening test will have an allergic reaction) and negative predictive value (NPV; the probability that subjects with a negative screening test will not have an allergic reaction). We used MedCalc statistical software to calculate the relative risk (RR), standard error, and 95% confidence interval (CI) for those exposed to HBAT, compared with the control group identified in the literature who received older botulinum antitoxin (pre-HBAT) preparations.

Outcome Definitions

Allergic reaction was defined by the Gell-Coombs classification of hypersensitivity as an immunoglobulin E (IgE)-mediated (type I), immediate hypersensitivity reaction, in which symptoms develop over minutes to hours, usually in <2 hours. Clinical symptoms include pruritus, urticaria, angioedema,

shortness of breath, cough, wheeze, hypotension, vomiting, and dizziness or fainting. Anaphylaxis was defined as a severe, potentially fatal, allergic reaction that was rapid in onset after contact with an allergy-causing substance [14, 15]. We reviewed all study reports found throughout the systematic literature review for symptom description in an effort to distinguish true allergy from adverse reaction. If a reaction was classified as “nonallergic” but met our criteria for possible allergy or anaphylaxis by description, we classified it according to the above definitions.

RESULTS

Search Results

Our search string terms captured 1883 citations related to allergy and 493 citations related to ST; after we deleted duplicate citations, there were 1109 and 307 citations, respectively. After reconciliation of the 2 searches, 1190 citations remained—883 related to allergy, 81 to ST, and 226 to both. These citations were screened by title and abstract for relevance. Eight additional articles were identified from hand-searching of references and communication with SMEs. A total of 356 full-text articles were assessed for eligibility. Articles were excluded for the following reasons: not botulinum antitoxin related, animal study, editorial or letters to the editor, guidelines, recommendations or case reports, basic science, review articles, absence of any primary data, were not in English, or were not relevant to allergic reactions or ST. For articles to be included, they had to contain

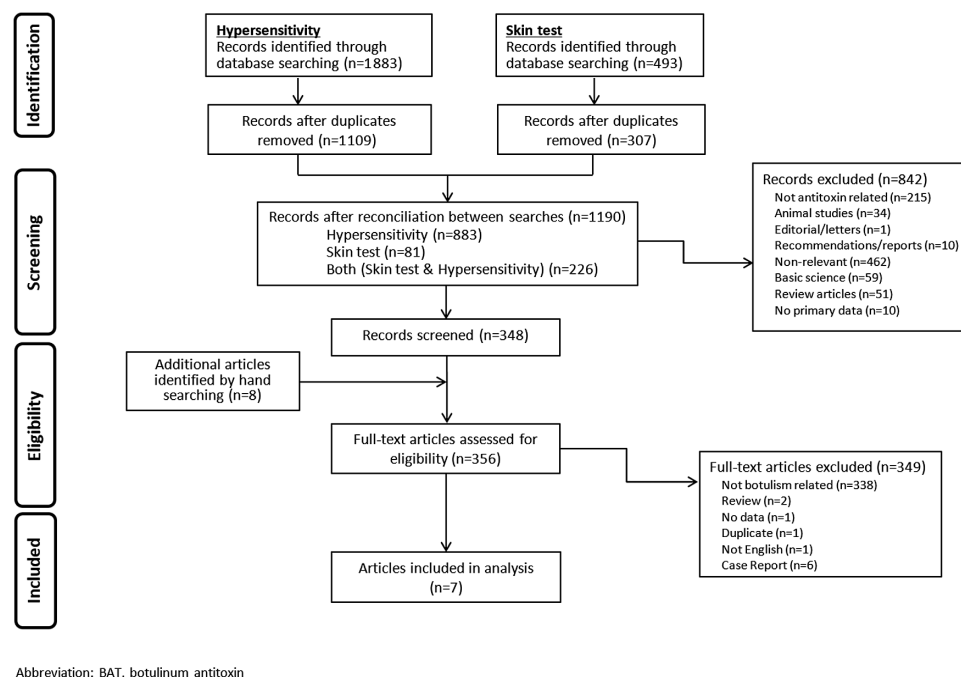


Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram: botulinum antitoxin (BAT) allergic reaction and skin testing literature review.

Table 2. Reviewed Studies: Allergic Reactions and Anaphylaxis in Persons Receiving Equine Botulinum Antitoxins, 1980–2016

Study (Author)	Year Published	Study Period	Equine Product Valency (Serotype)	Participant Age	Patients Treated	Allergic Reactions Reported	Anaphylaxis
Black and Gunn [17]	1980	1967–1977	Bivalent (AB) or Trivalent (ABE)	<20 y, n = 40; 21–30 y, n = 46; 31–40 y, n = 49; >40 y, n = 110; unknown, n = 23	268	14	5
Barrett [18]	1991	1973–1986	Trivalent (ABE)	Not reported	35	0	0
Roblot et al [22]	1994	1966–1990	Equine antitoxin, serotype not reported	5–70 y, average 32 y	74	1	0
Hibbs et al [21]	1996	1996	dBIG heptavalent (A–F), trivalent (ABE)	Not reported	54	8	0
Wongtanate et al [16]	2007	2007	Heptavalent (A–G), bivalent (AB), quadrivalent (ABEF)	17–74 y, average 44 y	91	16	2
Vanella de Cuetos et al [19]	2011	1993–2007	Bivalent (AB)	Infants <12 mo	31	4	0
Mottate et al [20]	2016	1962–2014	Monovalent (E), tetravalent (ABEF), unknown (n = 120)	0–20 y, n = 16; 30–59 y, n = 44; >60 y, n = 16; unknown, n = 63	134	1	1
HBAT data (clinical trial data and patient treatment data) ^a	Unpublished	2006–2013	HBAT (A–G)	10 d–88 y (15 pediatric patients included)	305	12	5

Abbreviations: dBIG, “despeciated” heptavalent botulism immune globulin; HBAT, heptavalent botulinum antitoxin.

^aCenters for Disease Control and Prevention (CDC) unpublished data. Includes 56 healthy adult subjects from 2 clinical trials by Cangene Corporation (2006–2009) and 249 botulism patients treated with HBAT under the CDC-sponsored expanded-access Investigational New Drug program (BB-IND 6750) (2010–2013).

information on the type of botulinum antitoxin and incidence of allergic reaction (Figure 1).

Allergic Reactions

Among the citations included in our report, 7 contained relevant information on allergic reactions (Table 2) [16–22] and 4 on ST (Table 3) [16, 17, 19, 21], which were combined for analysis. The total number of patients in the combined review was 687; most were adults. In one study, all 31 treated patients were infants; there were no reports of anaphylaxis [19]. Because not all articles specified individual patients’ ages, there were insufficient data to specifically comment on age as a predisposing factor (Table 2). Among 687 patients treated with

botulinum antitoxin, 45 (6.5%) allergic reactions occurred, including 8 (1.2%) anaphylaxis episodes (Table 2). The 7 articles did not contain data on risk factors for allergic reaction to botulinum antitoxin.

We compared the data from HBAT found in the literature review with those from manufacturer clinical trials and from treatment data supplied by CDC. Among 305 patients (56 from clinical trials and 249 from CDC antitoxin release data) who received HBAT, 12 (3.9%) allergic reactions occurred, including 5 (1.64%) anaphylaxis episodes. No significant difference in incidence of anaphylaxis was found between the systematic review data and the HBAT data: 1.16% vs 1.64%, respectively (RR, 1.4 [95% CI, .5–4.3]; $P = .5$) (Figure 2).

Table 3. Skin Test Results in Persons Receiving Equine Botulinum Antitoxin Products, by Study Identified in Systematic Review and by Product, 1980–2016

Study	Equine Product	No. of Skin Tests	Pos	Neg	Reactions in Pos	Reactions in Neg	PPV	NPV
Vanella de Cuetos et al [19] ^a	Bivalent (AB) (Aventis Pasteur)	31	3	27	3	1	Desensitized	96%
Hibbs et al [21] ^a	dBIG (extensively purified Fab’2)	50	6	44	6	0	Desensitized	100%
Black and Gunn [17]	Connaught (ABE), Connaught (E), Lederle (AB)	151	9	142	5	4	56%	97%
Wongtanate et al [16]	Heptavalent (A–G), bivalent (AB), quadrivalent (ABEF)	35	7	28	1	7	14%	75%
	Bivalent (AB)	29	5	24	0	5	0%	79%
	Quadrivalent (ABEF)	6	2	4	1	2	50%	50%
HBAT patient treatment data ^b	Cangene heptavalent (A–G)	33	1	32	1	0	100%	100%

For each study, the number of patients that had been skin tested prior to administration of botulinum antitoxin is shown. The PPV and NPV were calculated for each group.

Abbreviations: dBIG, “despeciated” heptavalent botulism immune globulin; HBAT, heptavalent botulinum antitoxin; neg, negative skin test result; pos, positive test result; NPV, negative predictive value; PPV, positive predictive value.

^aFor the Vanella de Cuetos et al and Hibbs et al studies, no PPV could be determined as all patients with a positive skin test were desensitized.

^bBotulism patients treated with HBAT under Centers for Disease Control and Prevention–sponsored expanded-access Investigational New Drug program (BB-IND 6750) (2010–2013).

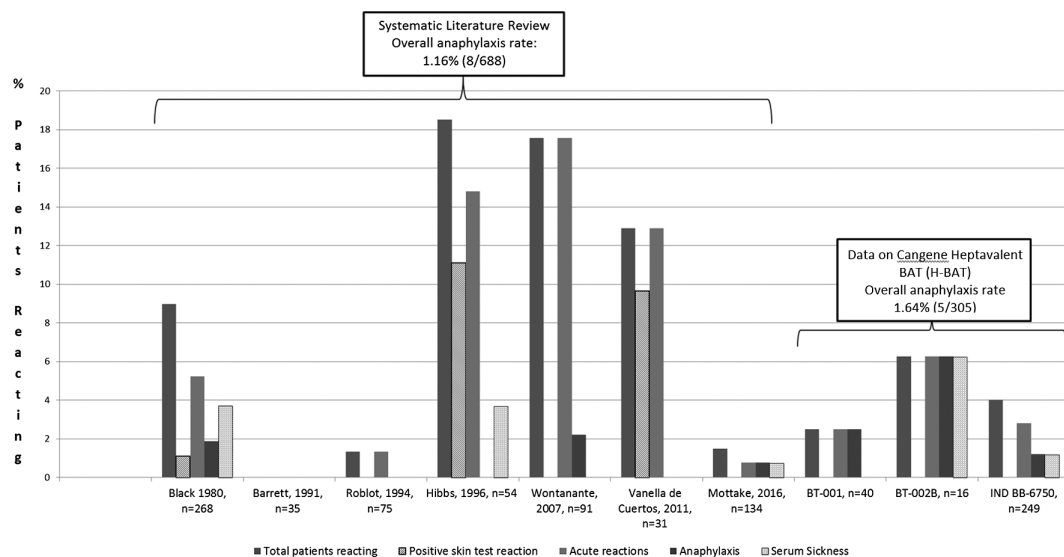


Figure 2. Systematic review: chronological presentation of allergic reaction rates in recipients (n) of equine botulinum antitoxin (BAT) products by study, 1980–2016. Allergic reactions included all reports of allergic reaction including anaphylaxis. For the clinical trial data, any reaction fitting the criteria of allergic or anaphylactic reaction was counted as such, even if not reported as allergic or anaphylactic in the trial report.

Skin Testing Predictive Value

Information from 4 studies was available for 302 patients who underwent ST [14, 15, 17, 19]. In 3 studies, the method of ST was noted as “intradermal” [17, 19, 21]; one study did not report the ST method [16]. Reported PPVs of ST in these studies ranged from 0 to 56% and NPVs ranged from 50% to 100%. In the HBAT database, ST information was available for 33 of 305 patients (approximately 10%), without details of ST methods. One patient was reported to have had a positive ST and was the only patient reported to have had an allergic reaction.

DISCUSSION

In this first, comprehensive systematic review of allergic reactions to botulinum antitoxin and the predictive value of ST before administration of antitoxin, we found that anaphylaxis rates for historical and currently licensed botulinum antitoxin formulations were each <2%. In efforts to determine the PPV of pretreatment skin testing to predict allergic reaction to botulinum antitoxin, we found few data on older forms of botulinum antitoxin, and limited data on HBAT.

Data on ST are extremely limited, so the accuracy of ST cannot be determined. The data on HBAT-ST are even more limited, with only 10% of treated botulism patients tested and only 4 of the 5 patients with anaphylaxis having been skin tested. The ST procedure is time consuming, and it is informative only when performed by a qualified, properly trained clinician. Most clinicians tending to botulism patients in a mass-exposure event would be unlikely to be adequately trained to administer and interpret ST. Given that administering the antitoxin as quickly as possible is the key to preventing progression of paralysis, using ST in a mass-exposure botulism event would not

be practical and therefore not advisable. Clinicians who treat patients with botulism still need to be prepared for the occurrence of anaphylaxis among 1%–2% of recipients. In all such situations, at minimum, epinephrine and antihistamine, and, to the degree possible, intensive care, should be available.

Equine-derived antitoxin has been in use for more than a century. It is produced by serially vaccinating horses with increasing doses of antigen until sufficient neutralizing antibody is produced in the serum. Animal proteins can be highly immunogenic to humans. In addition to the desired neutralizing antibody, animal immune serum contains a large load of non-specific, immunogenic serum proteins and immunoglobulins. These include IgA, IgD, IgM, IgE, and several subclasses of IgG. Neutralizing equine botulinum antitoxin is primarily composed of IgG₁, a highly glycosylated IgG that is particularly immunogenic in humans [23, 24]. Studies of equine antisera to venoms have shown that no more than 20% of equine antibodies in antitoxin are directed specifically against the target toxin, whereas <5% are neutralizing antibodies, some with overlapping neutralizing epitopes with different affinities [25]. This combination leads to a low activity-to-volume ratio, requiring higher doses [25]. The percentage of neutralizing antibody produced by the same mechanism to botulinum is likely similar. Early antitoxin products were minimally purified and frequently caused acute and delayed hypersensitivity reactions that were sometimes fatal. Antitoxin purification methods were developed throughout the 20th century. Advances include pepsin or papain digestion to extract the F(ab')₂ or Fab portion of the antibody, followed by salt or caprylic acid precipitation of allergenic equine carbohydrate moieties. Ion-exchange chromatography may be used to remove traces of contaminants [25]. In theory, these processes decrease

the potential for the products to induce an IgE-mediated response, though remnants of these additives may themselves induce reactions in susceptible individuals.

HBAT, the only US Food and Drug Administration (FDA)-licensed botulinum antitoxin currently available in the United States for noninfant botulism, is a heptavalent antitoxin with antibodies against 7 botulinum neurotoxin types (A–G) [26]. HBAT is derived from equine plasma and contains purified F(ab')₂/Fab immune globulin fragment products from horses vaccinated with botulinum toxoid and toxins. HBAT production entails despeciation by pepsin digestion, viral inactivation by tri-*N*-butyl phosphate (TNBP) and Triton X-100 (TX-100), and viral filtration. Residual amounts of pepsin, TNBP, and TX-100 remain in the finished product, each of which has the potential to induce an allergic response [27, 28, 29]. The final product contains F(ab')₂ and F(ab')₂-related fragments. The formulated antitoxin contains approximately 3–7 g% (30–70 mg/mL) protein [30]. While despeciation and purification reduce the antigenicity of botulinum antitoxin, inclusion of more serotypes in HBAT than earlier antitoxin preparations may increase its antigenicity by providing a greater number of potential antigens against which the recipient IgE antibodies can form.

Skin testing has been used in an attempt to identify patients likely to experience allergic reactions to equine-derived botulinum antitoxin. However, ST for drug allergy is complex, time consuming, and has predictive values that vary greatly by drug. It commences with a full-strength prick ST along with histamine and saline controls. If prick ST is negative after 15 minutes of observation, intradermal ST is performed. Proper application and interpretation requires training and practice. Full testing with both prick skin and intradermal methods would require at least 40 minutes when applied and reactions must be evaluated by a trained specialist.

For botulinum antitoxins predating HBAT, ST was recommended, and package inserts included instructions for skin-prick and intradermal testing, as well as administration instructions for sensitive patients (defined as those with history of allergy or sensitivity to horse serum and manifesting a positive reaction on ST). However, data to support those recommendations were not provided. Current nonbotulinum, FDA-approved, equine-derived products are also packaged with information on ST (eg, equine rabies immune globulin and Atgam, a lymphocyte immune globulin) or do not include a recommendation to ST (eg, Anascorp, an equine-derived scorpion F(ab')₂). When ST information is provided, it states that a negative ST to these products does not preclude an allergic reaction to the product.

To further evaluate the value of ST and risk of allergic and anaphylactic reactions to equine antitoxins, we reviewed reports of reactions to other equine antitoxins: antisnake venom and antirabies. Because snake venom itself can cause complement activation and anaphylaxis, it is not possible to differentiate anaphylaxis caused by venom and that caused by antitoxin among treated patients [31]. Intradermal ST to

snake and scorpion antivenoms have shown PPV averaging 32%, indicating that hypersensitivity ST is ineffective in predicting immediate-type hypersensitivity manifestations among patients given snake and scorpion antivenom [32]. Purified equine rabies antitoxin (pERIG), like botulinum antitoxin, is a highly purified (industrial chromatographic purification) equine immunoglobulin product that is digested with pepsin into F(ab')₂ fragments. In a case series in the Philippines, 7495 patients were skin tested using the intradermal method before administration of pERIG. The PPV was zero, and the rate of allergic reactions was 0.14% [33]. Due to poor predictive value of pERIG skin testing, elimination of ST before administration of pERIG has been proposed [34].

There are several important limitations to our study. Data were extracted and assessed only from English-language, retrospective case reports, and our conclusions rely on the completeness and accuracy of publications of widely varying periods, origins, detail, and quality. There was substantial heterogeneity in study designs, analyses, and treatment groups. Literature regarding botulinum antitoxin allergenicity is sparse and not specific to currently available HBAT. ST data were extremely limited, and the exact method of skin testing was not described in many reports. Some patients with positive ST [19, 21] were desensitized before receiving botulinum antitoxin. We classified desensitized patients as having positive reactions in the final analysis. Finally, the Cangene clinical trials excluded subjects who had known risk factors for adverse reactions, including allergies to horse, horse serum, or other horse products; severe food or seasonal allergies; asthma; positive horse allergy ST before dosing; previous treatments with commercial botulism neurotoxins; and abnormalities of nerve conduction. Subjects with positive ST results to HBAT were excluded from the 2 clinical trials we reviewed.

In conclusion, we found that data for estimating the allergic reactions and anaphylaxis rates in botulinum antitoxin recipients and the PPV of preadministration ST were sparse and not specific to the new HBAT. HBAT appears to have the potential to induce acute allergic reactions including anaphylaxis, similar to older preparations. Based on historical studies, the rate of anaphylaxis to botulinum antitoxin is estimated at 1%–2% and there are no reports of fatal anaphylaxis to botulinum antitoxin. Data on ST are extremely limited, and the accuracy of ST cannot be determined. The data on HBAT-ST are even more limited, with only 10% of treated botulism patients tested and only 4 of the 5 cases of anaphylaxis having been skin-tested. The ST procedure is time consuming, and it is informative only when performed by a qualified, properly trained clinician; most clinicians tending to botulism patients in a mass-exposure event are unlikely to be adequately trained to administer and interpret ST. Given that administering the antitoxin as quickly as possible is the key to preventing progression of paralysis, ST in a mass-exposure botulism event would be not practical and therefore not advisable.

Although not tested with botulinum antitoxin or HBAT specifically, it may be prudent to consider an approach commonly used with drug testing to minimize the risk of severe anaphylaxis. In such approach, the individuals with known potential risk factors for reaction including allergies to horse, horse serum, or horse products; severe food or seasonal allergies; asthma; positive horse allergy ST before dosing; previous treatments with commercial botulinum neurotoxins; and abnormalities of nerve conduction would be given a test dose—for example, 10% of the total dose of HBAT, followed by 90% of the dose after 20 minutes, if no reaction to the test dose was elicited. Treating physicians still need to be prepared for the occurrence of anaphylaxis in 1%–2% of HBAT recipients; at minimum, epinephrine and antihistamine, and, to the degree possible, intensive care, should be available.

Finally, it would be important to establish standardized reporting of adverse immune-mediated reactions to antitoxin, to allow more accurate assessment of the frequency and risk factors for adverse reactions to these products, as well as the long-term follow-up of patients who manifest hypersensitivity to HBAT to establish the natural course of the sensitization to HBAT constituents and any potential cross-reactivity with other products or horse allergens.

Notes

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