Effects of injection of botulinum toxin type B into the external anal sphincter on anal pressure of horses

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Objective—To determine effects on anal pressure of horses after local injection of the external anal sphincter with *Clostridium botulinum* toxin type B.

Animals—11 healthy adult horses.

Procedure—Peak and resting anal sphincter pressures were measured with a custom-made rectal probe that was connected to a pressure transducer. Pressures were measured before treatment and after injection with botulinum toxin type B (BTB) or saline (0.9% NaCl) solution. Dose titration with 500, 1,000, 1,500, and 2,500 U of BTB was completed. Physical changes, behavior, and anal pressure were recorded for each horse.

Results—Injection of 1,000 U of BTB caused a significant reduction in peak anal pressure from days 2 to 84, compared with pressure in control horses. Maximal effect of the toxin was observed within the first 15 days after injection, followed by a slow return to baseline during the 168-day period. Injection of 2,500 U of BTB in the anal sphincter in 1 horse resulted in lethargy, generalized weakness, and dysphagia for 14 days. Adverse clinical effects were not observed in horses after injections with 500, 1,000, or 1,500 U of BTB.

Conclusions and Clinical Relevance—The effect of focal intramuscular injection of BTB in horses is similar to that reported for other species. However, horses appear to be more sensitive to BTB, compared with other species, and clinical signs of botulism may develop at doses exceeding 1,500 U. Injections of BTB in the external anal sphincter of mares may be useful to reduce incisional dehiscence after repair of perineal lacerations. (*Am J Vet Res* 2004;65:26–30)

Toxins produced by the gram-positive bacteria *Clostridium botulinum* cause transient chemodenervation of mammalian striated muscles. The toxin binds to specific proteins within cholinergic presynaptic nerve terminals that regulate the release of acetylcholine in the synaptic space, resulting in loss of muscle activation and function. Duration of effect is dependent on regeneration of new motor end plates, which requires 2 to 4 months in mammalian species.[1] Local injections of botulinum toxins are currently used in humans for the treatment of disorders that may benefit from prolonged neuromuscular blockade, such as strabismus, blepharospasm, focal dystonia, spasticity, tremors, and anal fissures.[2,3]

Anal fissures in humans heal poorly as a result of increased tension of the fissure edges and decreased blood flow.[4] One or 2 injections of **botulinum toxin type A (BTA)** into the internal or external anal sphincter can cause relaxation of the anal canal and allow healing of chronic anal fissures.[4] Perineal lacerations in mares that occur during foaling often dehisce after surgical repair because of the high pressure across the incision resulting from accumulation of feces in the rectum.[5,6] We hypothesized that local injections of **botulinum toxin type B (BTB)** into the external anal sphincter of horses would cause a decrease in anal pressure, thus reducing the incidence of dehiscence when used before surgical repair of perineal laceration in mares.

The purpose of the study reported here was to determine the effects of BTB injection in the external anal sphincter in clinically normal horses. Our hypothesis was that local injection of BTB would result in transient reduction of anal tone without causing adverse clinical effects.

Materials and Methods

Animals—Eleven healthy adult horses were used in the study. Horses included 1 Thoroughbred mare and 10 geldings (2 Thoroughbreds, 3 Quarter Horses, 2 Warm Bloods, 2 Paint horses, and 1 Arabian). Body weight ranged from 488.6 to 676.4 kg (mean, 586.2 kg), and age ranged from 7 to 24 years (mean, 12.2 years). The horses did not have a history of vaccination with a *C botulinum* toxoid for at least 1 year before the study. All horses were maintained on pasture for the duration of the study, except for the experimental period when they were restrained in stocks for injection of BTB[a] and measurement of anal tone. Water and sweet feed were offered to the horses during the measurement period to evaluate their ability to swallow. The experimental procedures used in this study were approved by the Virginia Tech Animal Care Committee.

Experimental procedure—A probe designed to measure anal sphincter tone was made from a cuffed endotracheal tube[b] that was modified by inserting 2 rubber rings at the tip of the endotracheal tube to maintain the cuff within the anal canal during measurements. Mean length of the anal canal in horses[7] is 5 cm; therefore, the length of the cuff and the distance between the rings was 5 cm. The endotracheal tube was connected to an electronic pressure transducer[c] and monitor[d]

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to measure anal sphincter pressures. The electronic pressure transducer was calibrated to a mercury manometer before and after the experiments. For each horse, the pressure transducer was calibrated to a value of zero when the cuff was filled with 10 mL of water before each of the measurements. Horses were restrained in stocks, and peak and resting anal sphincter pressures were measured. Peak pressures were recorded when reflex constriction of the anal sphincter was induced by slight rotation of the rectal probe. Peak and resting anal pressures were recorded before treatments were administered (baseline). Five measurements of peak and resting anal pressures were obtained from each horse during the period when horses were standing quietly in the stocks. Mean value for the 5 measurements was used for data analysis.

Once baseline measurements were obtained, the horses were sedated by administration of xylazine hydrochloride[e] and restrained by application of a nose twitch. The perianal region was thoroughly scrubbed with chlorhexidine soap solution.[f] Horses were randomly assigned to treatment groups. Syringes that contained 4 mL of sterile saline (0.9% NaCl) solution (control horses) or 4 mL of BTB (1 of 3 doses; treated horses) were prepared for each of the experiments. Investigators were unaware of the treatment administered to each horse. Injection of the BTB or saline solution was accomplished by inserting 2 fingers into the anus to stretch the dorsal part of the anal sphincter caudally. A 22-gauge, 1.5-in needle was fully inserted (ie, to the level of the hub) in the external anal sphincter muscle. Contents of the syringe were slowly injected as the needle was gradually withdrawn from the muscle. Three separate experiments were performed to evaluate the effect of IM injection of BTB in the external anal sphincter.

Experiment 1—Two horses were used in this experiment. A single dose (2,500 U of BTB [4.4 U/kg]) was injected at the 12 o'clock position in the external anal sphincter in 1 horse (day 0). A control horse received a similar injection of an equal volume of saline solution. Anal pressures were measured on days 1, 2, 3, 4, 7, 8, 9, 10, 11, 12, 14, 17, 21, 28, 42, 56, 70, 84, 98, 112, 126, 140, 156, and 168 in the horse treated with BTB and days 1, 2, 3, 4, 7, 8, 9, 10, 11, 12, 14, 17, 21, 28, and 42 in the control horse. Swallowing reflexes and tone of the anal sphincter, tail, upper eyelid, and tongue were monitored daily for the first 28 days; subsequently, they were monitored at the time of anal pressure measurements. Temperature, heart rate, and respiratory rate were measured at the time of anal pressure measurements for the first 21 days.

Experiment 2—Three horses received injections of BTB. One horse received 500 U of BTB (0.88 U/kg), the second horse received 1,000 U of BTB (1.75 U/kg), and the third horse received 1,500 U of BTB (2.20 U/kg). A fourth horse served as a control animal and received sterile saline solution. Each dose was administered as two 2-mL injections at the 10 and 2 o'clock positions in the external anal sphincter of each horse. Anal pressures were monitored on days 1, 2, 3, 4, 5, 6, 7, 14, 21, 28, 42, 56, 70, 84, 98, 112, 126, 140, and 168. Swallowing reflexes and tone of the anal sphincter, tail, upper eyelid, and tongue were monitored. Temperature, heart rate, and respiratory rate were measured for the first 3 days after the injections.

Experiment 3—Three control horses, including the control horse from experiment 1, received sterile saline solution, and 3 horses received 1,000 U of BTB (1.70 to 2.05 U/kg) in the external anal sphincter. The injection protocol was the same as for experiment 2. Anal pressures were monitored on

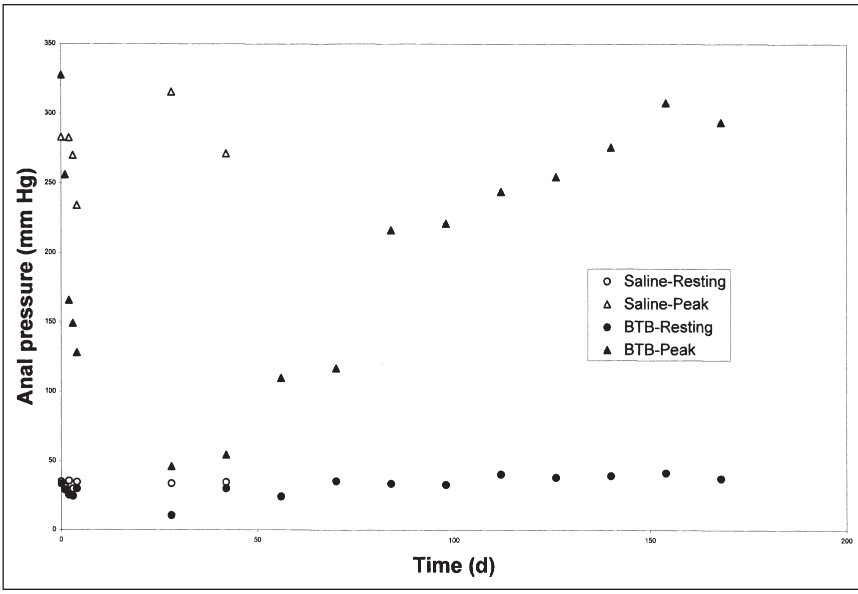

Figure 1—Resting and peak anal pressures in 2 horses in experiment 1. One horse received saline (0.9% NaCl) solution, and the other horse received 2,500 U of botulinum toxin type B (BTB) injected locally into the external anal sphincter. Day 0 = Day of injection.

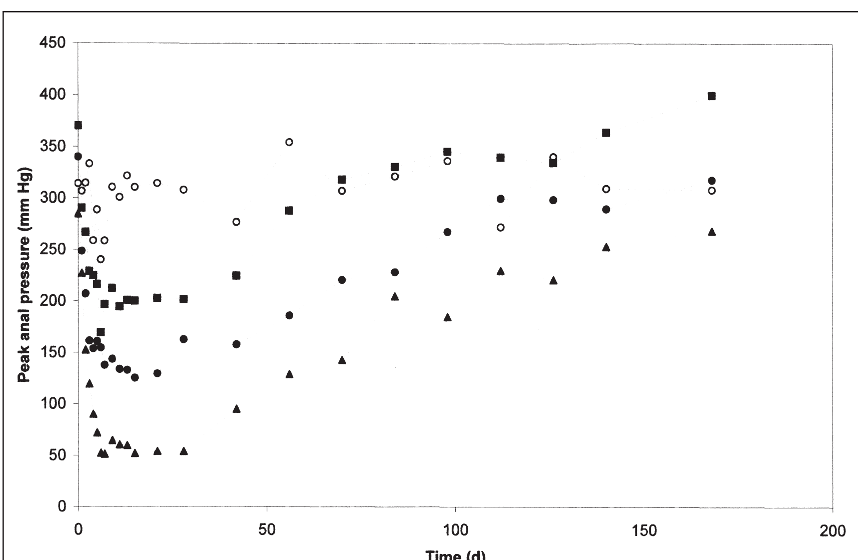

Figure 2—Peak anal pressures in 4 horses in experiment 2. Each horse received saline solution (open circle), 500 U of BTB (square), 1,000 U of BTB (triangle), or 1,500 U of BTB (solid circle) injected locally into the external anal sphincter.

days 1, 2, 3, 4, 5, 6, 7, 14, 21, 28, 42, 56, 70, 84, 98, 126, 140, 154, and 168. Horses were monitored as in experiment 2.

Statistical analysis—Because peak anal pressure at baseline differed among horses, results were compared on a percentage basis. A statistical program[g] was used to test the influence of treatment on values obtained on each day after treatment (repeated-measures ANOVA). The Bonferroni correction was applied. A value of $P < 0.05$ was considered significant.

Results

Experiment 1—The horse injected with 2,500 U of BTB had a 49.4% reduction of peak anal pressure on day 2 and a maximal reduction of 89% on day 11, compared with the baseline value (**Fig 1**). Peak anal pressure returned to 94% of baseline peak anal pressure on day 154. There was no change in the peak anal pressure in the control horse. On day 10, the horse that received BTB had mild generalized weakness, low head carriage, diarrhea, and dysphagia (evidenced as water reflux from the nostrils when drinking, prolonged chewing, hypersalivation, and partial dropping of grain). Signs of dysphagia and all other neuromuscular deficits had resolved by day 24. On day 4, the dorsal hemicircumference of the anus in the horse treated with BTB was extremely thin, as determined by digital palpation, compared with the thickness of the ventral portion of the sphincter. Normal sphincter thickness returned gradually during 98 days after BTB injection. We did not detect complications at the injection site in either horse.

Experiment 2—Horses treated with BTB had a mean reduction in peak anal pressure of 37.8% and 56.3% on days 2 and 5, respectively, compared with baseline peak anal pressure (**Fig 2**). The greatest mean decrease in peak anal pressure (63.6%) was detected on day 15. On day 168, mean peak anal pressure had returned to 98.6% of baseline peak anal pressure. The horse injected with 1,000 U of BTB had the greatest decrease in peak anal pressure (day 2, 46.4%; day 5, 74.8%; and day 15, 81.7%). On days 2 and 4, three fourths of the dorsal circumference of the anus in horses receiving 500 or 1,000 U of BTB was extremely thin, compared with the thickness of the ventral portion of the sphincter in those horses. Normal sphincter thickness gradually returned 56 to 84 days after injection of BTB. The horse receiving 1,500 U of BTB did not develop a distinct area of thinning in the anal sphincter in the area of the injections. None of the horses injected with BTB developed adverse clinical effects or had local reactions at sites of injection.

Experiment 3—Horses appeared to have a variable response to the same dose of BTB (**Fig 3**). Horses injected with BTB had a mean reduction in peak anal pressure of 46.7% and 60.3% on days 2 and 5, respectively, compared with baseline peak anal pressure (**Fig 4**). The greatest decrease in peak anal pressure (68%) was observed on day 15. On day 168, mean peak anal pressure had returned to 96.2% of baseline peak anal pressure. Peak anal pressure differed significantly between BTB-treated horses and control horses from days 2 to 84. Peak anal pressure of BTB-treated horses was lower, but not significantly different from, peak anal pressure of control horses from days 84 to 140. Resting anal pressure did not change after BTB or saline injections. On days 2 and 3, three fourths of the dorsal circumference of the anal sphincter was extremely thin, compared with the thickness of the

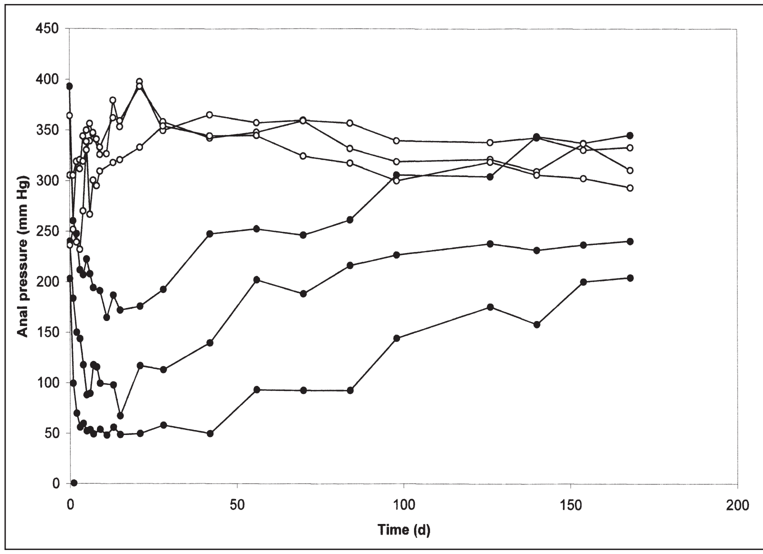

Figure 3—Peak anal pressures in each of 6 horses in experiment 3. Horses received saline solution (open circle; 3 horses) or 1,000 U of BTB (solid circle; 3 horses) injected locally into the external anal sphincter.

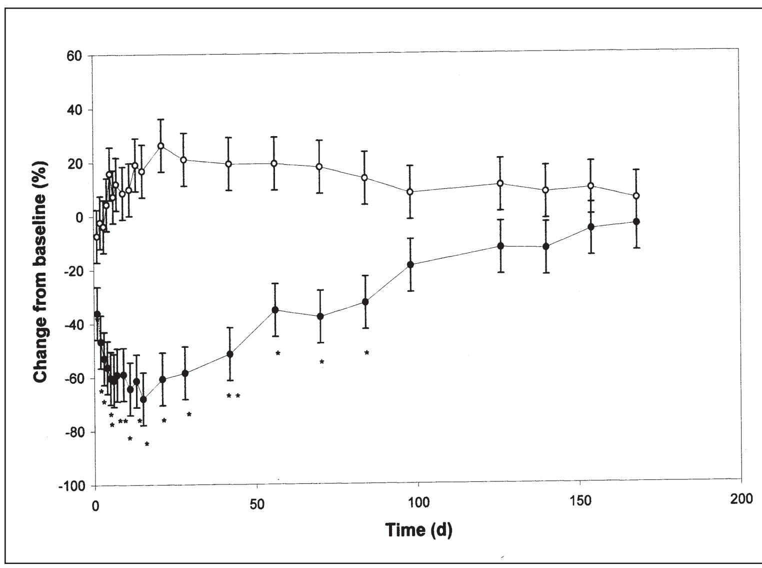

Figure 4—Mean ± SD percentage change from baseline peak anal pressure after local injection of saline solution (open circle; 3 horses) or 1,000 U of BTB (solid circle; 3 horses) into the external anal sphincter in experiment 3. *Within each day, values differ significantly ($P < 0.05$) between BTB-treated and control horses.

ventral portion of the anal sphincter, in all 3 BTB-treated horses. Normal sphincter thickness gradually returned 56 to 84 days after treatment. None of the horses injected with BTB developed adverse clinical effects or had local reactions at sites of injection.

Discussion

Seven types of *C botulinum* neurotoxins have been identified (ie, A, B, C, D, E, F, and G).[1] The mechanism of action of botulinum toxin is in 4 steps. Toxin binds to presynaptic membranes of peripheral nerve terminals. Toxin is then internalized into endocytic vesicles, leading to the formation of an acidic environment within vesicles. Toxin is activated as a result of a conformational change controlled by the acidic environment, followed by translocation of the L-chain portion of the toxin into the cytoplasm. Finally, there is catalysis of **soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE)** proteins.[1] The SNARE proteins regulate vesicular exocytosis of neurons. Proteolytic cleavage of SNARE proteins prevents fusion of acetylcholine vesicles with the plasma membrane, resulting in blockade of neuromuscular transmission and muscle paralysis.[8] Histologic changes observed in muscles injected with *C botulinum* toxin include the conservation of anatomic contact with nerves without apparent loss of motor axons and the accumulation of small synaptic vesicles on the cytosolic side of the plasma membrane.[9] During renewed neuromuscular transmission, electrical activity from nerves causes presynaptic and postsynaptic production of neurotrophins that induce nerve sprouts around the motor end plate, resulting in the formation of new nerve fibers from a single motor axon. There is also enlargement of the motor end plate and spreading of acetylcholinesterase and acetylcholine receptors in other areas of the muscle plasma membrane.[1,9] During a second phase of repair, there is total regeneration of the original motor end plates and elimination of nerve sprouts.[10]

In humans, BTA is currently used for the treatment of chronic anal fissures.[11-14] One or 2 injections of BTA into the internal or external anal sphincter causes relaxation of the anal canal and allows healing of chronic anal fissures, presumably as a result of improved blood flow and decreased tension on the fissure edges.[4] In 1 study,[3] injections of BTA into the internal anal sphincter caused a reduction in resting anal pressure (26% and 29% reduction 1 and 2 months after treatments, respectively. Injection of BTA in the external anal sphincter in humans induces relaxation of the internal and external anal sphincters by local diffusion of the toxin.[14] In the study reported here, peak pressure, but not resting pressure, was significantly decreased in BTB-treated horses. The internal anal sphincter is a continuation of the rectal wall and responsible for maintaining resting anal pressure, whereas the external anal sphincter is a separate thick circular layer of striated muscle responsible for voluntary peak anal contraction. Therefore, it would be expected that toxin injections in the large external anal sphincter of horses would primarily affect voluntary (peak) anal pressures but not resting anal pressures. Alternatively, our rectal probe may not have been sufficiently sensitive to detect subtle changes in resting anal pressure.

Doses of 2,500 to 10,000 U of BTB are currently recommended as an effective and safe treatment for cervical dystonia in humans. The most common adverse effects are dry mouth (3% to 34% of treated patients) and dysphagia (16% to 25% of treated patients). Adverse effects are the result of local diffusion of the toxin, and they are more common when higher doses of toxin are used.[15] In the study reported here, 2,500 U of BTB injected in the external anal sphincter of 1 horse caused clinical signs of botulism, including generalized weakness, lethargy, and dysphagia. Lack of toxic effects when 500, 1,000, or 1,500 U of BTB was injected suggested that these amounts are safe for injection as a split dose at 2 sites in the external anal sphincter.

Ingestion of *C botulinum* spores or preformed toxin causes the clinical syndrome of botulism in horses, which is characterized by generalized muscle weakness; low head carriage; mild lethargy; slowness when drinking or eating; dysphagia; decreased tone of the tongue, eyelid, and tail; mydriasis; recumbency; and colic. In severe cases, death may result from respiratory paralysis or complications associated with prolonged recumbency.[16,17] The rate of progression of clinical signs after ingestion of contaminated feed depends on the dose of toxin, and the condition is usually fatal unless treated promptly with the specific antitoxin. Horses are most commonly affected by type B and less commonly by type C or A. An effective toxoid vaccine is available for *C botulinum* type B in horses.[16,17] It is not known whether horses would respond to local injections of BTB after vaccination with the toxoid.

Injection of BTB (500 to 2,5000 U) in the external anal sphincter of horses caused a reduction in peak anal pressure in all horses, compared with pressure in control horses. Maximal effect of the toxin was observed within the first 15 days after injection, followed by a slow return to baseline during the 168-day period. Onset of muscle relaxation after local injection of BTB for treatment of cervical dystonia in humans is evident within a few days to 1 week, and peak effect is observed within 2 weeks after injection.

Individual variation was also observed in experiment 2, with the horse injected with 1,000 U of BTB having a greater percentage decrease in peak anal pressure, compared with values for the horse injected with 1,500 U. This was an unexpected finding because a similar or greater reduction of muscle function would be expected with increasing doses of BTB. The significant change in peak anal pressure in 3 horses injected with 1,000 U of BTB in experiment 3 suggested that this dose has a local effect for approximately 3 months. This duration is similar to the duration of muscle relaxation in humans, which may last for 3 to 4 months after injections.[15]

Our study was initiated with the idea that BTB could be injected into the external anal sphincter of mares prior to surgical repair of perineal lacerations. Dehiscence of perineal incisions as a result of pressure from accumulation of feces in the rectum is a commonly reported complication after surgical repair.[5,6] The decreased muscle tone after BTB injection may allow repair of the anal sphincter at the same time the rectovaginal shelf and perineal body are repaired, rather than requiring veterinarians to use a 2-stage technique.[5] Because the peak effect of BTB in horses was observed 15 days after injections, it would appear logical that BTB should be injected at least 15 days before surgery to repair perineal lacerations. In the study reported here, a single injection of BTB at the 12 o'clock position caused a reduction of peak anal pressure, but the ventral portion of the anal canal retained normal muscle thickness, which indicated lack of diffusion of the toxin throughout the entire anal sphincter. Injections at the 10 and 2 o'clock positions that were intended to achieve diffuse relaxation of the entire anal canal did not achieve relaxation of the most ventral portion of the anal sphincter. Because perineal lacerations occur at the ventral portion of the anal canal, it would appear prudent to inject equal amounts of BTB at 4 equidistant sites in the external anal sphincter.

Local injections of BTB in the external anal sphincter of horses caused transient relaxation of the anus and reduction of peak anal pressures. Adverse systemic effects were observed only in 1 horse that received the highest dose of BTB (2,500 U), which suggested a narrow dose range to avoid toxic effects. Additional research is needed to test the effects of botulinum toxin in clinical cases and determine the full potential of this treatment modality.

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[a]Myoblock, Elan Pharmaceuticals, San Francisco, Calif.
[b]Aire Cuff, 24-mm outside diameter, Bivona Inc, Gary, Ind.
[c]Gould P23 ID, Viggo-Spectramed, Oxnard, Calif.
[d]Datascope 2000, Datascope Corp, Paramus, NJ.
[e]Sedazine, Fort Dodge Animal Health, Fort Dodge, Iowa.
[f]Nolvasan solution, Fort Dodge Animal Health, Fort Dodge, Iowa.
[g]SAS, release 8.2, SAS Institute Inc, Cary, NC.

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