Chapter 17
Botulinum Toxin Treatment in Veterinary Medicine: Clinical Implications

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Abstract Botulinum toxin (BoNT) products are not licensed for veterinary use, but there are studies investigating its therapeutic potential in veterinary medicine, mainly in dogs and horses. Some efficacy has been reported for BoNT in the treatment of osteoarthritic and perioperative pain in dogs and in the treatment of lameness in horses in small controlled clinical trials. In addition, few case series have described the use of BoNT in the treatment of lower esophageal sphincter achalasia-like syndrome, urinary incontinence, and prostatic hypertrophy in dogs and in stringhalt in horses. Further thoroughly planned controlled clinical trials with objective outcome measures are needed to reveal the true relevance of BoNT in veterinary medicine.

Keywords Botulinum toxin injection · Canine pain therapy · Equine movement disorders · Intra-articular treatment

In contrast to human medicine, the therapeutic potential of botulinum toxin (BoNT) is not fully exploited in veterinary medicine, and BoNT products are not licensed for veterinary use. Conditions characterized by constant painful muscle overactivity, such as dystonias, are rarely seen or treated in animals, and the toxin has mainly been a concern among veterinary professionals due to unwanted events, where spoiled foliage has led to the death of many animals or whole packs [1–3].

However, BoNT has potential in pain therapy of veterinary patients, especially in companion animals. The direct antinociceptive effect of BoNT has been studied in the treatment of osteoarthritic and postoperative pain in dogs, and some evidence supports its use for pain therapy in this species. Additionally, the chemodenervation produced by BoNT might benefit laminitic equine patients in the future.

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BoNT in the Treatment of Canine Osteoarthritis

Osteoarthritis (OA) is considered the leading cause of lameness and chronic pain in dogs. Estimates on its prevalence vary from 2.5% to 20% [4, 5]. In a recent UK study, OA was estimated to affect 200,000 dogs annually [5]. OA causes significant discomfort and pain and impairs the quality of life of the affected animals. As one of the most common reasons for euthanasia in dogs [6], OA also impacts lifespan, especially in working animals [7]. Multimodal treatment consisting of exercise modification, weight management, physiotherapy, nutraceuticals, and pain medication is recommended for OA treatment in dogs. In addition, some osteoarthritic canine patients are eligible for joint prosthesis. The requirement for oral analgesics in osteoarthritic dogs may be lessened by intra-articular (IA) treatment, which directly targets the painful joint.

IA-injected botulinum neurotoxin A (BoNT-A) has shown some efficacy in the treatment of osteoarthritic pain in dogs. Hadley et al. (2010) were the first to describe the effects of IA BoNT-A in dogs [8]. They conducted a pilot study lasting 12 weeks on five client-owned dogs with elbow or hip OA. All dogs received an IA injection of 25 U of onabotulinumtoxinA (Botox, Allergan Inc., USA) into the osteoarthritic joint. The response to treatment was assessed by measuring the ground reaction forces, i.e., weight-bearing, with a pressure platform. In addition, the owners graded their dog's locomotion and discomfort.

The ground reaction forces of the treated limbs improved in all dogs for a variable period of time, but remained inferior to those of the contralateral limbs, implying that the dogs remained somewhat lame. Two owners reported significant improvement, while moderate improvement, mild improvement, or no change was reported in the other three dogs at the end of the study. A mild increase in lameness in addition to redness and swelling over the injected joint was detected in two dogs. No other adverse events were detected during the study.

Although this was a small preliminary study without any control group, the improvement detected in the ground reaction forces was encouraging. There are no direct ways to measure pain in animals, and therefore, canine pain evaluation is based on the lack of normal behavior or on the presence of pain-associated behavior such as lameness. Measuring weight-bearing is an objective, quantitative, and unbiased method to evaluate lameness in dogs [9, 10].

The efficacy of IA BoNT-A injections in the treatment of chronic osteoarthritic pain was further investigated by Heikkilä et al. in 2014 in a placebo-controlled, randomized, double-blinded clinical study on 35 client-owned osteoarthritic dogs with chronic lameness due to OA in the stifle, elbow, or hip joint [11]. The dogs were randomized to receive either an IA injection of 30 U of onabotulinumtoxinA or placebo (saline) into the painful osteoarthritic joint. The primary outcome variables were ground reaction forces measured with a force plate and the Helsinki

Chronic Pain Index (HCPI), a questionnaire for dog owners validated for the evaluation of chronic canine orthopedic pain [12]. The subjective pain score evaluated by a veterinarian and the need for rescue analgesia were used as secondary outcome variables. The study lasted 12 weeks.

In BoNT-A-treated dogs, a significant improvement was detected in the ground reaction forces at the end of the study (week 12), while no change was observed in the dogs treated with placebo (Fig. 17.1). There was also a significant improvement from baseline in the HCPI of the dogs treated with BoNT-A, but not in the dogs

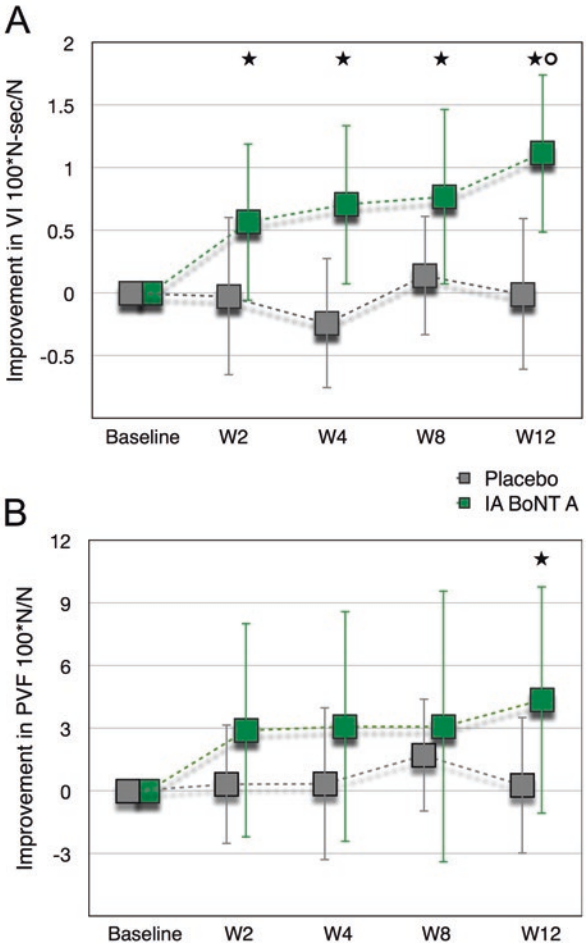

Fig. 17.1 Improvement from baseline in vertical impulses (**a**) and peak vertical forces (**b**) (mean and 95% CI) after intra-articular botulinum toxin A ($n = 16$) or intra-articular placebo ($n = 15$) in osteoarthritic dogs. Baseline, before the injections; IA BoNT A intra-articular botulinum toxin A; placebo, 0.9% saline, PVF peak vertical force, VI vertical impulse, W week. $^{\circ}P \leq 0.005$ between groups; $\bigstar P \leq 0.05$ within group. (Reprinted from the Heikkilä et al. [11], Elsevier (2014), with permission from Elsevier)

treated with placebo. The duration of the treatment effect could not be evaluated, since the effect was the largest at the end of the study.

No severe adverse events were detected. One dog developed a superficial skin infection over the injected hip joint 1 week after BoNT-A injection, and another one developed a mild disc protrusion during the study.

A more recent study by Nicacio et al. in 2019 investigated the efficacy of another botulinum toxin A preparation, IA abobotulinumtoxinA (Dysport, Ipsen Pharmaceuticals, Ireland), in the treatment of hip OA in 16 client-owned dogs [13]. Dogs with moderate or severe hip OA due to hip dysplasia were enrolled in the study. The dogs were randomized to receive an IA injection of either 25 U of BoNT-A or saline serving as control.

The response to treatment was assessed by owner and veterinary evaluations for 90 days. The owner evaluation included the HCPI and the Canine Brief Pain Inventory (CBPI) questionnaires, both validated for the evaluation of chronic pain in dogs [14].

Improvement from baseline was detected in HCPI, CBPI, and veterinary evaluation in both the treatment and the control groups. However, there was no significant difference between the two groups in any of the outcome measures at any time point during the study. Four dogs in the treatment group and one in the control group experienced local adverse events, not further specified, in the first 24 hours after the IA injection. No severe systemic adverse events or local muscle weakness were detected.

The conflict among the results of these studies may be explained by the fact that the dosages of onabotulinumtoxinA and abobotulinumtoxinA are not interchangeable. The different preparations of BoNT-A produced by different manufacturers differ in biological potency [15]. Conversion ratios of 4:1 and 3:1 for abobotulinumtoxinA (Dysport) and onabotulinumtoxinA (Botox) have been suggested for human patients suffering from cervical dystonia [15, 16], but this conversion ratio has not been evaluated in BoNT pain therapy or in dogs. Nevertheless, the lack of clinical efficacy in the study by Nicacio et al. might be explained by the smaller biological potency of the product. In addition, veterinarians and pet owners are prone to detect improvement in osteoarthritic dogs after any treatment, including placebo [17]; therefore, veterinary and owner assessments, including the validated owner questionnaires, are susceptible to a caregiver placebo effect. Objective outcome measures such as weight-bearing measurements may reveal mild treatment effects, which might not be detectable using only subjective veterinary or owner evaluations. Pressure platforms and force plates can detect very subtle changes in weight-bearing not visible to the naked eye. The drawback of these methods is that there is no consensus on what magnitude of improvement indicates clinically meaningful pain relief in dogs.

Despite several studies on IA BoNT in human patients [18], there is not much information on the possible adverse effects of the toxin inside the joint. Therefore,

Heikkilä and colleagues aimed to investigate whether the toxin affects the canine cartilage and whether it spreads from the joint after the IA injection [19]. They conducted a longitudinal, placebo-controlled, randomized clinical trial in six healthy laboratory Beagle dogs. The dogs were randomized to receive an IA injection of 30 U of onabotulinumtoxinA into the right or left stifle joint. An equivalent volume of saline serving as placebo was injected into the contralateral joint. The dogs were evaluated for clinical and cytological adverse effects and for spread of the toxin for 12 weeks. After 12 weeks the dogs were euthanized, the injected joints and the adjacent muscles and nerves were evaluated histologically, and autopsy was performed.

No clinical, cytological, or histological adverse effects were reported during the study. The electrophysiological recordings showed low compound muscle action potentials in two dogs in the BoNT-A-injected limb, suggesting that the toxin had spread from the joint. However, the clinical impact of such spread seemed to be low because the abnormalities detected in the electrophysiological recordings were not associated with any clinically meaningful neurological deficit. Autopsy and histopathological examinations of the joint and adjacent muscles and nerves did not reveal changes associated with IA BoNT-A.

BoNT as Adjuvant Surgical Pain Treatment in Dogs

Many dogs not intended for breeding are neutered. In addition, dogs undergo surgery for orthopedic and traumatic conditions and for neoplasia. Surgery in veterinary medicine has become less traumatic and invasive, and many procedures can be performed laparoscopically. On the other hand, especially in veterinary oncology, more extensive and complex surgeries are being performed. Meanwhile, perioperative pain management has greatly developed in recent years. The understanding of pain in animals and its consequences on the patients has deepened, and the monitoring of anesthesia has improved considerably, due to the availability of better equipment characterized by a broader spectrum. This has led to the use of a wider range of analgesic agents and methods. Current perioperative pain management can be a complex combination of constant-rate infusions and sedative, inductive, and inhalation agents, in addition to local analgesia and nerve blocks and non-steroidal anti-inflammatory drugs.

It is not surprising, in this context, that also BoNT injections have been studied in the treatment of perioperative pain in dogs. Vilhegas et al. (2015) conducted a placebo-controlled, randomized, blinded study on the efficacy of BoNT-A injections in the treatment of perioperative pain [20]. Sixteen client-owned, middle-aged to old bitches of various breeds and sizes with malignant mammary gland tumors requiring bilateral chain mastectomy were enrolled in the study. The dogs were

randomized to receive either a total dose of 7 U/kg of abobotulinumtoxinA divided into each mammary gland or injections of sterile saline as control. The injections were performed in the middle of each mammary gland 24 hours before surgery. Postoperative pain was evaluated by the modified Glasgow Composite Measure Pain Scale (modified-GCMPS) and the visual analogue scale (VAS) up to 72 hours after surgery. The modified-GCMPS is a validated questionnaire for veterinary professionals to evaluate postoperative pain in dogs based on pain-associated behavior [21]. Rescue analgesia was administered depending on the modified-GCMPS and VAS scores.

BoNT-A injections appeared effective in reducing postoperative pain, as the modified-GCMPS and VAS scores were significantly lower in the BoNT-A group compared with the control group. In addition, the need for rescue analgesia differed between the groups: In the BoNT-A group, two out of eight dogs needed rescue analgesia (two doses of rescue analgesia in total), compared with seven out of eight dogs in the control group (17 doses in total). The histopathological tumor classification, the number and size of the nodules, and the degree of inflammation did not differ between the groups. No adverse events were noted during the study, which ended at the time of suture removal, 10–14 days after the surgery.

This study presents a promising addition to multimodal perioperative pain therapy in dogs undergoing bilateral chain mastectomy, or possibly other invasive surgeries. In this study, the dogs were premedicated with BoNT-A injections into the center of the mammary gland 24 hours before surgery, although in a similar study on human breast cancer patients the toxin was injected intramuscularly during surgery [22]. Layeeque et al. proposed that the pain-relieving efficacy of BoNT-A injections in their study was mediated by the inhibition of pectoralis muscle spasms. In the study by Vilhegas et al., the mechanism of action was suggested to be the inhibition of neuropeptide release from afferent nociceptive nerve endings. Because the mammary glands were removed in the surgery, the toxin probably exerted its effects in the central nervous system rather than in the periphery. BoNT molecules have been shown to undergo retrograde transport via the axon from the peripheral nerve ending into the cell soma and to bridge synapses while preserving their activity [23, 24].

Bringing the dog to the clinic for premedication before surgery might be inconvenient for some dog owners. However, premedication with BoNT-A could be considered as an adjuvant pain therapy, especially for dogs in which nonsteroidal anti-inflammatory drugs are contraindicated.

Application of BoNT as Paralytic Agents in Dogs

Paralytic agents are seldom used in veterinary patients. Conditions leading to painful muscle overactivity are rare, and severely disabled animals are euthanized to spare them further suffering. There are no controlled studies on the paralytic effects

of BoNT in animals, but a few case series and case reports have been published. In addition, two case series have exploited the toxin's anticholinergic effects in the treatment of lower urinary tract disease and ptyalism in dogs.

A recent retrospective case series described the use of BoNT-A in the treatment of lower esophageal sphincter achalasia-like syndrome (LES-AS) in 14 client-owned dogs [25]. The main clinical sign was regurgitation, and almost all the dogs had megaesophagus. A condition resembling human lower esophageal achalasia was diagnosed. All dogs were treated with mechanical dilatation of the lower esophageal sphincter following injections of BoNT-A. A total of 32 U of onabotulinumtoxinA was injected in the lower esophageal sphincter area. The dogs were presented for follow-up at a median of 21 days after treatment. The body weight of the dogs had markedly increased, the frequency of regurgitation reported by the owner was significantly reduced, and all owners reported subjective clinical improvement. Megaesophagus was not resolved and there were no changes in esophageal motility, but gastric filling had improved, explaining the clinical improvement. However, the median duration of the effect was only 40 days. Six dogs were further surgically treated. Two complications were reported after BoNT-A injections. One dog developed aspiration pneumonia and another developed gastroduodenal-esophageal intussusception and hiatal hernia requiring surgical treatment.

BoNT-A injections combined with mechanical dilatation thus appeared to be effective in the treatment of dogs suffering from LES-AS, but the short duration of the effect, which would require repeated procedures, was considered disappointing. The authors suggested that the response to BoNT treatment could be used to select the LES-AS patients which would benefit from surgery and that repeated BoNT injections could be used to allow the animals to grow before the definitive surgical treatment. It is not known how much of the improvement was due to the BoNT-A injections rather than to mechanical dilatation.

Three case reports describe the use of BoNT as a paralytic agent in dogs. Rogatko et al. (2016) reported a case in which repeated BoNT-A injections were successfully used for the treatment of neuromyotonia and myokymia in a dog [26]. The case was a five-year-old Maltese dog suffering from persistent muscle contractions and involuntary continuous muscle activity in the right thigh after receiving radiation therapy. The condition was refractory to conventional treatment. The affected muscles were injected with a total dose of 24 U of onabotulinumtoxinA, resulting in the resolution of the clinical signs in 10 days. The injections were successfully repeated at 3- to 4-month intervals for more than a year without adverse effects.

Another case report describes the use of BoNT injections to treat severe myoclonus in a 13-month-old mixed-breed midsized dog suffering from canine distemper encephalomyelitis [27]. It had developed tetraparesis and severe, debilitating myoclonus 8 months after the owner had found it in poor condition. After several other treatment methods had failed, a total amount of 100 U of onabotulinumtoxinA was injected into the most affected muscles. The procedure was repeated with 140 U of BoNT-A 18 days afterwards, after which the clinical signs subsided for several

months: The dog was reported to be ambulatory and able to run long distances 180 days after the injections. The dog had an episode of hyperthermia and weakness of the thoracic limbs 15 days after the second injection, which were thought to be adverse events caused by the toxin. However, the weakness rapidly resolved within 2 days.

Rinaldi et al. (2014) described a case in which onabotulinumtoxinA injection was used to treat delayed gastric emptying in an Australian Shepherd which had developed functional gastric outflow obstruction after several surgeries due to bile leakage and peritonitis [28]. A total amount of 400 U of onabotulinumtoxinA (91 U/kg) was injected into the pylorus in a laparoscopically assisted procedure. Both the dog's condition and its gastric emptying were improved after the injections, but euthanasia due to pancreatitis was performed 11 days afterwards. Pancreatitis was most likely a consequence of the primary condition of the dog, but diffusion of the toxin into the pancreas could not be excluded. Despite the final undesirable outcome, the authors argued that BoNT-A injection as a potential therapeutic modality for pyloric spasm warrants further investigation.

Treatment of blepharospasm was one of the first indications for BoNT injections in medicine [29]. Despite this, only one case report describes the use of BoNT injections to treat this condition in a dog [30]. A total amount of 200 U of abobotulinumtoxinA was injected into the orbicularis oculi muscle of both eyes of a 3-year-old Great Dane suffering from bilateral essential blepharospasm refractory to conventional treatment. Improvement in the condition was evident within 3 days, and the spasms were reported to have completely disappeared 6 days after treatment. In the following 3 years, the dog received repeated injections at 3- to 4-month intervals.

In addition to dogs, one case report is available in which BoNT injections were used to treat congenital right hind limb arthrogryposis in a cat (2007) [31]. An 11-week-old cat was presented to a veterinarian for congenital right tarsal deformity and non-weight-bearing lameness. The cat received 20 U of onabotulinumtoxinA into the spastic right gastrocnemius muscle. Despite this treatment, the cat did not start to bear weight on the limb, and the condition was then successfully treated with surgery. This was the first report to describe the use of BoNTs in cats.

BoNT Injections in Lower Urinary Tract Disorders in Dogs

BoNT injections are considered effective in the treatment of lower urinary tract disorders such as neurogenic detrusor overactivity and non-neurogenic overactive bladder in human patients [32, 33]. The effects of intramuscularly injected BoNT in the bladder are thought to be produced by inhibition of the nociceptive and parasympathetic pathways, because its receptor and intracellular target proteins are not expressed in urothelial or bladder muscular cells [34].

The use of BoNT injections in dogs with lower urinary tract disease has been described. Lew et al. published a prospective case series in which BoNT injection was used to treat urinary incontinence in 11 client-owned bitches in 2010 [35]. The dogs suffered from clinical urinary incontinence with no detectable underlying reasons. The dogs represented various breeds and were aged 2–8 years. Nine of the dogs were neutered. The dogs were treated with 50–100 U of onabotulinumtoxinA, depending on the size of the animal. The toxin was injected submucosally into the bladder wall in a cystoscopic procedure. The evaluation of the treatment effect was left to the dog owners. One dog did not respond to treatment, while urinary incontinence decreased in all the other dogs, for a variable time period, in their owners' assessment. The duration of the treatment effect ranged from 1 to 13 months, the average being 5 months. Although controlled studies with objective outcome measures should be conducted, BoNT injections might provide an alternative treatment for dogs suffering from urinary incontinence refractory to conventional treatment.

A case series describing the effect of intraprostatic BoNT injections in the treatment of benign prostatic hyperplasia in dogs [36] is also available. Eight client-owned, intact, midsized, and middle-aged male dogs were included in the study. All dogs had clinical signs of benign prostatic hyperplasia such a hematuria, urethral bleeding, or constipation, and their prostate was enlarged. A total 250 U of onabotulinumtoxinA was injected into the prostate of the dogs, equally divided between the two lobes. The treatment effect was evaluated up to 16 weeks after treatment. In addition, semen was collected before and after the procedure.

Urethral bleeding resolved in all dogs and hematuria in all but one. The duration of the effect was not reached in the 16-week study. Two dogs that suffered from constipation before the injection did not show clinical improvement regarding this clinical sign. The prostatic diameter or volume did not change significantly from the baseline values. Interestingly, the treatment had no effect on the libido of the dogs, nor on the quality of their semen. Two dogs were allowed to mate successfully after the injection. No abnormalities were detected in the following pregnancy, gestation duration, or litter size.

Benign prostatic hyperplasia is a very common condition among older intact male dogs, affecting 80% of those over 5 years of age [37]. It is best treated by castration, although androgen suppression therapy is also commonly used if castration is declined by the dog owner or if anesthesia is contraindicated. This case series suggests that BoNT injection might be considered an alternative treatment for breeding male dogs suffering from benign prostatic hyperplasia. However, in a recent meta-analysis in human patients, BoNT injection showed no benefit over placebo in the treatment of benign prostatic hyperplasia in men, and the clinical efficacy of BoNT injections detected in previous studies has been attributed to a marked placebo effect [38].

One paper presents a dog in which severe ptyalism was successfully treated with BoNT-A injections into both mandibular salivary glands [39]. The dog was an

11-year-old Collie with ptyalism due to difficulty in swallowing because of esophageal adenocarcinoma. Ptyalism was reported to be decreased after the injections for the 12 weeks until the animal was euthanized. However, assessing the treatment effect of the toxin is difficult, because in addition to BoNT injection, an esophageal stent was placed at the same time to improve swallowing and relieve the mass effect produced by the carcinoma in the esophageal lumen.

BoNT injections have also been studied in dogs for application to human therapy of several disorders, including the induction of ptosis [40] and cricothyroid muscle paralysis [41], the reduction of prostatic contractility [42] and parasympathetic activation of the heart [43], the inhibition of biliary leakage [44], and the reduction of salivary gland [45] and nasal secretions [46].

BoNTs in Equine Veterinary Medicine

A few publications describe the use of BoNT in equine medicine. From the veterinary point of view, equids and companion animals differ in the aim of the treatment. In addition to reducing the amount of suffering of the individual animal, the aim of treatment in equids is often to fully recover the previous level of performance. Not reaching this aim might lead to economic loss for the owner and euthanasia of the animal. Perhaps the most promising studies investigate BoNT as adjuvant to laminitis pain therapy in horses, and one controlled study exploits the direct antinociceptive effects of BoNT in the treatment of horse lameness.

Laminitis is a common debilitating condition in equids, affecting approximately 1.5–24% of the equine population [47] and resulting in economic loss in the horse industry and discomfort and pain, lameness, loss of performance, and euthanasia of the affected animals. For long, laminitis was considered a dreaded consequence of severe systemic inflammation or, more rarely, of mechanical overload on the affected limb [48]. However, endocrinopathies such as pituitary pars media dysfunction and hyperinsulinemia associated with equine metabolic syndrome have recently been shown to be the leading causes of laminitis in equids [49]. Laminitis is characterized by the disruption of the lamellar tissue between the distal phalanx and the epidermis of the keratinized hoof wall. In a healthy animal, this lamellar region attaches the distal phalanx to the hoof capsule, resisting the pull of the deep digital flexor tendon attached to the caudal aspect of the distal phalanx. The disruption of this tissue results in pain, separation of the distal phalanx from the hoof wall, and displacement of the distal phalanx inside the hoof capsule [50, 51].

Equine laminitis remains a therapeutic challenge for veterinarians. The aim of the treatment is to treat the underlying causative factor, provide analgesia, and prevent further lamellar damage and displacement of the distal phalanx. Treatment depends on the underlying etiology and includes diagnosis and treatment of the underlying cause, pain and anti-inflammatory medication, exercise restriction, digital hypothermia, therapeutic orthotics and shoeing, and dietary modification [52].

Deep digital flexor tendon tenotomy has been reported to provide pain relief and improve prognosis in horses with chronic laminitis refractory to medical treatment [53]. The purpose of this procedure is to reduce the pull of the deep digital flexor tendon on the distal phalanx and prevent its displacement. With a similar aim, Carter and Reinfoe (2009) published a case series of seven laminitic horses in which the deep digital flexor muscle was chemically denervated with BoNT injections [50]. The horses were client-owned, suffering from acute or chronic laminitis, and of various ages and breeds. They received injections of 100–200 U of onabotulinumtoxinA into the deep digital flexor muscle of either one or both front limbs. The horses' response to treatment was followed for a period ranging from 6 weeks to 3 years. The injections resulted in improvement in the condition of six of the seven horses, most becoming pasture-sound and one becoming pain-free during riding in all gaits. One horse was euthanized 6 weeks after the injections because of persistent pain. No adverse events were reported.

The effects of BoNT-A on the deep digital flexor muscle were further investigated by both Wijnberg and Hardeman in 2013 [54, 55]. They showed with quantitative needle electromyography that BoNT-A injections reduce the activity of the deep digital flexor muscle in healthy horses, without systemic toxicity. In addition, Hardeman et al. reported that such chemodenervation does not cause lameness or change the weight distribution in the hoof of healthy horses, as iatrogenic gait abnormalities would prevent the use of this novel treatment in laminitis. There is some evidence of increased muscle force in the deep digital flexor muscle of laminitic ponies and horses [56]. Thus, reducing this force with BoNT injections might provide a safe, noninvasive, and reversible adjuvant treatment of laminitis. However, the clinical efficacy of this treatment remains to be investigated in a controlled prospective study in laminitic equine patients.

Wijnberg has also studied the efficacy of BoNT injections in two Dutch warmblood dressage horses suffering from stringhalt in 2009 [57]. Stringhalt is an uncommon horse gait abnormality characterized by the spasmodic hyperflexion of one or both tarsi while walking [58]. Systemic anticonvulsants have been proposed as a medical treatment, and surgical treatment consisting of lateral digital extensor tendon myotenectomy has resulted in improvement [59, 60]. Wijnberg and colleagues injected a total amount of 700 U of onabotulinumtoxinA into the hind limbs of the two horses in four separate occasions in 28 days. Hyperflexion and adduction were reduced in the affected hind limbs for approximately 12 weeks, but the gait abnormality was not totally abolished.

In addition to these results, the effect of a different BoNT serotype, botulinum neurotoxin B (BoNT-B), has been studied on anal pressure in healthy adult horses [61]. Reducing the anal tone is thought to be beneficial in the repair of perianal lacerations in mares after parturition. Seven horses received injections of rimabotulinumtoxinB (Myobloc, Solstice Neurosciences, USA) to their external anal sphincter and five received saline injections as control. One horse received 2500 U, while the others received 500–1500 U of BoNT-B. Anal pressure was monitored with a custom-made probe for up to 168 days after the injection. The treatment resulted in a 38–89% reduction in anal pressure, depending on the amount injected.

The greatest reduction was measured in the horse receiving 2500 U (4.4 U/kg), 15 days after treatment, after which anal pressure gradually increased to normal levels in 151 days. However, the same horse developed clinical signs of generalized botulism 10 days after the injection, including generalized weakness, low head carriage, diarrhea, and dysphagia, which resolved 24 days after the injection. The other horses did not experience clinical adverse effects.

Although BoNT injections reduced the anal pressure in healthy horses in this study, no studies have investigated how much BoNT injections benefit mares suffering from perineal lacerations. This study emphasizes the fact that generalized botulism may be a concern when using BoNT injections in horses, which are among the species most sensitive to botulism [62].

Two controlled studies investigated the direct pain-relieving effect of BoNT injection in horses. Gutierrez-Nibeyro and colleagues (2013) published a study on BoNT-B injections in the treatment of lameness due to degenerative injury of the podotrochlear apparatus in 2014 [63]. The podotrochlear apparatus consists of the navicular bone and the associated soft tissue structures in the hoof region. Injury to these structures can result in acute or chronic front limb pain. Oral and intra-articular anti-inflammatory drugs, controlled exercise, corrective shoeing, and extracorporeal shockwave therapy have been used for the treatment of chronic lameness due degenerative injury to the podotrochlear apparatus [64, 65]. Still, the majority fail to recover their previous level of performance [65]. Interestingly, the pain in the soft tissue structures of the podotrochlear apparatus is mediated by nerve fibers containing substance P, calcitonin gene-related peptide, and neurokinin-A [66], all neuropeptides inhibited by BoNT. In the study by Gutierrez-Nibeyro et al. (2013), seven client-owned Quarter Horses suffering from chronic, bilateral, degenerative injury to the podotrochlear apparatus received an injection of BoNT-B into the navicular bursa. The limb with more severe lameness was treated, while the ipsilateral limb was not injected and served as control. RimabotulinumtoxinB at 3.8–4.5 U/kg was injected into the navicular bursa. The response to treatment was evaluated by veterinarians assessing lameness from video recordings in random order over 14 days. Lameness severity significantly decreased from baseline in the treated limbs. However, despite this improvement, the horses remained lame. The authors speculated that this might have resulted from a too small dosage of BoNT-B or the fact that the pain did not arise exclusively from the navicular bursa. The control limbs were not injected, and therefore, it is not certain whether the reduction in lameness was produced by BoNT or by the injection itself.

In addition, the antinociceptive efficacy of IA BoNT-A has been studied in acute synovitis in four healthy experimental horses with somewhat surprising results [67]. Two horses received 50 U of onabotulinumtoxinA into the middle carpal joint of both limbs, while two horses serving as controls received injections of saline. Acute synovitis was induced with interleukin-1 β (IL-1β) injection into one of the injected joints of each horse 14 days afterwards, while the other injected joint served as control and received an injection of saline. The antinociceptive efficacy of BoNT-A was evaluated by veterinary evaluation and by a computer-assisted kinematic analysis of lameness after the IL-1β injection. The horses were euthanized 15 days after the start of the study and the injected joints were histopathologically evaluated.

Both the control horses developed prominent front limb lameness after the IL-1β injection. Interestingly, only one of the BoNT-A-treated horses developed lameness, while the other remained sound. Suppurative inflammation was detected in the histopathological examination of the synovia in all IL-1β-injected joints. No abnormal findings were noted in the joints injected with BoNT-A but not IL-1β. No adverse events were detected during the study.

The results of this study were surprising, as one horse responded to BoNT-A very well, while the other did not respond, although both had developed synovitis after the IL-1β injection. The discrepancy in the treatment response was not further explained in this study due to the small sample size.

Conclusion

Only a few controlled studies and some case series have assessed the benefit of BoNT injections in veterinary medicine, and these are summarized in Tables 17.1 and 17.2. As so often in this discipline, the number of animals in these studies is small, and many include only subjective outcome measures. The veterinary clinician might be tempted to extrapolate study results from human medicine. However, different species differ in sensitivity to different BoNT serotypes [68]. Even in the same species and with a single BoNT serotype, different biological potency has been reported between different BoNT preparations provided by different manufacturers [15]. Therefore, further thoroughly planned controlled clinical trials with objective outcome measures are needed to reveal the true relevance of BoNT in veterinary medicine.

Table 17.1 Controlled clinical trials on BoNT in veterinary patients

Category	Animals	Treatment	Control	Outcome measures	Study period	Results
Treatment of OA pain in dogs [13]	16 client-owned dogs with hip OA BoNT-A group: Age 6.3 Y (3.9 Y) Mean (SD) Weight 25.1 kg (12.7 kg) Control group: Age 4.6 Y (2.3 Y) Weight 24 kg (7.8 kg)	IA injection of 25 U of BoNT-A (Dysport) *N* = 8	IA injection of saline *N* = 8	HCPI, CBPI, veterinary evaluation	12 W	No difference between groups in improvement in HCPI or CBPI No adverse events

(continued)

Table 17.1 (continued)

Category	Animals	Treatment	Control	Outcome measures	Study period	Results
Postoperative pain treatment in dogs [20]	16 client-owned dogs with mammary gland tumors BoNT-A group Age 8.75 Y (3 Y) Mean (SD) Weight 13 kg (8 kg) Control group: Age 16 Y (12 Y) Weight 9.5 kg (2 kg)	Injection of 7 U/kg of BoNT-A (Botox) into mammary glands *N* = 8	Injection of saline into mammary glands *N* = 8	Modified GCMPS, VAS, rescue analgesia	10–14 D	Significantly less pain in BoNT-A group compared to control group No adverse events
Treatment of OA pain in dogs [11]	35 client-owned dogs with chronic stifle, hip, or elbow OA Age 6.3 Y (3.2 Y) Mean (SD) Weight 33.1 kg (8.8 kg) Various breeds	IA injection of 30 U of BoNT-A (Botox) *N* = 16	IA injection of saline *N* = 15	Ground reaction forces, HCPI, veterinary evaluation, rescue analgesics used	12 W	Significant improvement in BoNT-A group compared to control group and baseline. Local skin infection over injection site 1/35 dogs, disc protrusion 1/35 dogs
Treatment of chronic pain in horses [63]	7 client-owned horses with bilateral degenerative injury to podotrochlear apparatus Age 11 Y (5–14 Y) median (range) Weight 553 kg (490–590 kg)	Injection of 3.8–4.5 U/kg of BoNT-B (Myobloc) into the navicular bursa *N* = 7	Injection of saline into the navicular bursa, contralateral limb *N* = 7	Veterinary evaluation of lameness from video recordings	14 D	Significantly less lameness in BoNT-A treated limbs compared to saline-treated limbs No adverse events

BoNT-A botulinum toxin A, *CBPI* Canine Brief Pain Inventory, *D* day, *HCPI* the Helsinki Chronic Pain Index, *IA* intra-articular, *modified-GCMS* modified Glasgow Composite Measure Pain Scale, *OA* osteoarthritis, *VAS* visual analogue scale, *W* week, *Y* year

Table 17.2 Case series on BoNT in veterinary patients

Category	Study method	Animals	Treatment	Outcome measures	Study period	Results
Treatment of ME and LES-AS in dogs [25]	Retrospective	14 client-owned dogs with ME and LES-AS Age 2 Y (0.9–5.8 Y) median (IQR) Various breeds	Injection of 32 U of BoNT-A (Botox) into the esophageal sphincter and mechanical dilatation	Clinical severity evaluated by owner, BW, BCS, regurgitation frequency, VFSS parameters	3.5 M (2–4.8 M) median (IQR)	Clinical severity, BW, and BCS improved, regurgitation decreased in all dogs, gastric filling improved in 12/14 dogs No improvement in ME. Duration of effect 40 D (17–53 D) median (IQR) Aspiration pneumonia in 1/14 dogs, intussusception and hiatal hernia in 1/14 dogs
Treatment of prostatic hypertrophy in dogs [36]	Prospective	8 client-owned intact male dogs with benign prostatic hypertrophy Age 5.8 Y (2.1 Y) mean (SD) Weight 18.4 kg (8.2 kg)	Injection of 250 U of BoNT-A (Botox) into the prostate	Clinical signs evaluated by owner, prostatic size and volume evaluated by radiography, ultrasonography, and retrograde urethrocystography, semen analysis, serum DHT and testosterone concentration	16 W	Clinical signs resolved or decreased in all dogs No significant change in prostatic size or volume Semen quality was preserved No change in DHT or testosterone concentration No adverse events

(continued)

Table 17.2 (continued)

Category	Study method	Animals	Treatment	Outcome measures	Study period	Results
Treatment of urinary incontinence in dogs [35]	Prospective	11 client-owned bitches with urinary incontinence Age range 2–8 Y 9/11 neutered Various breeds	Injection of 50–100 U of BoNT-A (Botox) into the bladder wall	Clinical signs evaluated by owner, hematology, serum biochemistry, urine analysis	1–13 M	Urinary incontinence resolved for a variable time period in 10/11 dogs Mean duration of treatment effect 5 M No adverse events
Treatment of chronic OA pain in dogs [8]	Prospective	5 client-owned dogs with elbow or hip OA Age 11 Y (5–15 Y) median (range) Weight 27.1 kg (1.7–43.0 kg)	IA injection of 25 U of BoNT-A (Botox)	Ground reaction forces, clinical signs evaluated by owner	12 W	Variable improvement in ground reaction forces in all dogs Improvement in owner evaluation in 4/5 dogs Mild redness and pain over injection site in 2 dogs
Treatment of laminitis in horses [50]	Not specified	7 client-owned horses with laminitis Age 12 Y (8–23 Y) Median (range) Various breeds	Injection of 100–200 U of BoNT-A (Botox) into the deep digital flexor muscle in one or both front limbs	Obel grading by veterinarian	6 W–3 Y	Pain decreased and Obel grading improved in 6/7 horses 1/7 horses euthanized No adverse events

Treatment of stringhalt in horses [57]	Prospective	2 dressage horses with neurogenic, idiopathic stringhalt in one hindlimb Age 6 Y and 3 Y Weight 565 kg and 637 kg	Injection of total amount of 700 U of BoNT-A (Botox) into hindlimb muscles in 4 occasions within 28 D	Veterinary evaluation Automated kinematic gait analysis system Semiquantitative sEMG analysis	12 W	Stringhalt signs decreased in both horses Reduction in sEMG signals Duration of effect 12 W

BCS body condition score, *BoNT-A* botulinum neurotoxin A, *BW* body weight, *D* day, *IA* intra-articular, *LES-AS* lower esophageal achalasia-like syndrome, *ME* megaesophagus, *M* month, *OA* osteoarthritis, *sEMG* surface electromyography, *VFSS* videofluoroscopic swallow study, *W* week, *Y* year

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