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Clinical observations of tetanus toxin plus decoy, Snoretox-1, a novel targeted neuromuscular stimulant, in a pilot study of 6 British bulldogs with BOAS

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ABSTRACT

Objective: To evaluate the novel therapeutic Snoretox-1 for the treatment of Brachycephalic Obstructive Airway Syndrome (BOAS) in British bulldogs.
Methods: A therapeutic (Snoretox-1) was developed consisting of a muscle-toning protein (tetanus toxin) and an antibody trap (decoy), previously demonstrated via in vivo studies to functionally increase local muscle tone in the presence of neutralising antibodies. British bulldogs with grade 2 or 3 BOAS were treated, under sedation, by injection of the therapeutic bilaterally into the centre of the rostral geniohyoid. Bulldogs were graded by an accredited unblinded observer utilising the Respiratory Functional Grading (RFG) scale, after a three-minute trot test, at multi-week intervals, until any improvement returned to baseline values.
Results: Six British bulldogs were enrolled in this study (two male, four female) aged between 4 and 8 years old. All dogs improved by at least one BOAS grade on the RFG scale. Non-parametric statistical analysis (Friedman χ^2 with Durbin-Conover post-hoc tests) showed that BOAS severity grades recorded after Snoretox-1 treatment at time points up to 12 weeks were significantly lower than after placebo ($p < 0.001$), with improvements lasting from 20 to 53 weeks post-treatment. Feeding difficulty for up to 5 ½ weeks may occur with incorrect placement outside the rostral geniohyoid.
Conclusions: Injection of the Snoretox-1 muscle-toning therapeutic decreased the severity of BOAS in British bulldogs by one grade or more for a period of between 20 weeks and 53 weeks.
Clinical Relevance: Snoretox-1 could offer a less invasive, well-tolerated and effective treatment for BOAS.

Key Points

- BOAS is an important cause of breathing difficulty, reflux, pneumonia, heatstroke and premature mortality in dogs.
- Current treatments may be inadequate.
- Snoretox-1, a potential new therapeutic treatment has been developed for treating BOAS based on tetanus toxin plus an antibody binding decoy.
- Six bulldogs received geniohyoid dosage of 25 Units TeNT/kg of Snoretox-1, resulting in statistically significant improvements in BOAS Respiratory Function Grading (RFG): $p < .001$.

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Introduction

Brachycephaly, derived from the ancient Greek words “brachy” (short) and “kephalē” (head), is the terminology that has been adopted for ‘brachycephalic’ dogs (Ita et al., 2023; Mitze et al., 2023). In recent times such brachycephalic breeds have had a resurgence in popularity due to their ‘snub-nose’ or ‘flat-faced’ appearance that owners find endearing, along with their small stature and low levels of aggression (Archer and Monton, 2011; Packer et al., 2017, 2019). This has also been driven by their popularity with celebrity influencers, in social media and in advertising (Ghirlanda et al., 2013; Herzog, 2006). In 2022, the world’s largest registry of purebred dogs, the American Kennel Club, reported French bulldogs had the most registrations at over 100,000 per annum, with British bulldog 6th, Boxer 16th, Boston Terrier 24th and Pugs 35th (Haid, 2023).

Selective breeding for extreme brachycephaly has given rise to significant health complications including Brachycephalic Obstructive Airway Syndrome (BOAS) (Krainer and Dupré, 2022; Oechtering et al., 2010; Serpell, 2003). BOAS is the result of a compressed and abnormal upper airway anatomy due to shortening of the skull without a proportional reduction in the soft tissues of the head (Hendricks, 1992). As negative inspiratory pressure in the chest increases, the upper airway collapses when soft tissues (e.g., everted laryngeal saccules and tonsils, thickened soft palate) are pulled into the airway, impeding airflow (Fasanella et al., 2010; Krainer and Dupré, 2022). Clinical signs associated with BOAS include loud breathing sounds, increased respiratory effort and rate, exercise intolerance, hyperthermia, episodes of regurgitation, sleep apnoea, cyanosis, collapse and death (Fasanella et al., 2010; Nationwide Pet Insurance, 2023). Amongst extreme brachycephalic breeds (such as British bulldogs, pugs and French bulldogs) 45–50% have clinically significant signs of BOAS (Ladlow et al., 2018). This leads to a lifetime of struggling to breathe, while clinical signs of BOAS are 'normalised' and accepted as 'part of the breed' by owners and even some veterinary professionals (Kenny et al., 2022; Packer and O’Neill, 2021; Packer et al., 2012; Sandøe et al., 2017). Despite dog owners recognising clinical signs of BOAS in their animals (Roedler et al., 2013; Schroers and Meyer-Lindenberg, 2022), such symptoms of BOAS are considered normal for brachycephalic breeds by approximately 75% of owners surveyed (Kenny et al., 2022). Additionally, dogs that suffer from BOAS are at significantly increased risk for a range of other conditions. For example, such dogs are twice as likely to have spinal disease, 4.5 times more likely to have pneumonia, and 5 times more likely to exhibit oesophageal disease (Nationwide Pet Insurance, 2023). As a result, the mean life expectancy of Grade 3 (the most severe grade) brachycephalic dogs is up to 4 years less than the less severe grades (O’Neill et al., 2015; Reich et al., 2023). Concern regarding the health of such animals has led to calls to stop the breeding of extreme brachycephalic breeds and a petition calling for the introduction of mandatory breathing tests for all British bulldogs, French bulldogs and pugs that are to be bred from (Fawcett et al., 2019; Gray, 2023).

There are different methods to assess airway function in these breeds. One technique developed by Bernaerts et al. (2010) and by The Kennel Club/Cambridge BOAS research team involves an objective non-invasive respiratory test using whole-body barometric plethysmography (Ladlow et al., 2018). These are custom devices but have limited practical utility in clinical settings due to expense and limited availability. Another technique is the Respiratory Functional Grading (RFG) scheme, a 4-point functional grading system based on listening to the airway before and after a short three-minute trot (6.4–8 km/h), designed to stress the upper airway. It includes auscultating the airway before and after the 3-minute trot test, and assessors evaluating two abnormal noises: stertor, which is a lower-pitched noise caused by airflow obstruction between the nose and the nasopharynx; and stridor, implying airflow obstruction from the opening of the larynx to the extra-thoracic trachea. Dogs exhibiting no noise (Grade 0) to mild stertor (Grade 1; only audible with a stethoscope) are considered clinically unaffected, while dogs with moderate (Grade 2; intermittent and audible without a stethoscope) to severe noise (Grade 3; loud, constant, audible without a stethoscope) are considered clinically affected. When comparing the plethysmography data with the respiratory functional grading scheme, the two assessments correlate well, with 94% agreement in determining BOAS affected and unaffected dogs (Ladlow et al., 2018). The Kennel Club (UK and Australia) use The Kennel Club/University of Cambridge's Respiratory Function Grading Scheme to assess British bulldogs, French bulldogs and pugs for BOAS, and it is a validated BOAS assessment that can be practically applied in both clinical and field research settings (“Dogs Australia, Respiratory Function Grading Scheme,” 2023; “The Kennel Club, Respiratory Function Grading Scheme,” 2023). Other submaximal exercise tests include the 6-minute walk test and the 1,000-m walk test, both of which show graded associations with clinically assessed BOAS severity (Aromaa et al., 2019; Lilja-Maula et al., 2017). Research is ongoing to develop other objective measures that can be utilised in a clinical setting (Gallman et al., 2023; Mach et al., 2022; Oren et al., 2023).

The treatments currently available for BOAS include surgery as well as medical management (Mitze et al., 2023), such as avoiding stressors, minimising exercise especially in the heat, weight loss, sedatives, and addressing comorbid aerodigestive disorders. Surgery is an invasive technique (Wallace, 2024), with several procedures currently in use, including turbinectomy, staphylectomy, excision of everted laryngeal saccules, partial arytenoidectomy and arytenoid lateralisation (Fawcett et al., 2019). The mortality risk from such procedures has been reported to be as high as 7% (Ree et al., 2016; Riecks et al., 2007). This problem has driven the development of preoperative scales to predict the risk of major complications or death in dogs undergoing corrective surgery for BOAS (Tarricone et al., 2019). However, post-surgically, dog’s respiratory function remains compromised in up to 60% of cases (Liu et al., 2017). Only 8.7% of eligible dogs (i.e. Grade 2 or 3 BOAS) present for BOAS surgery (private correspondence from insurance company executive). There are many reasons for why owners do not choose surgery, including the 15% risk of a major surgical complication, and a mortality rate of 7%, as described by Nanda and Hans (2025). There is clearly a growing need for a safer, less invasive and effective therapeutic treatment for BOAS.

Snoretox-1: a potential new pharmacological treatment for BOAS

Over the last two decades, formulations of botulinum neurotoxin (BoNT; Dysport/Botox) have become a widely used and highly effective pharmaceutical treatment offering symptomatic relief across a wide spectrum of neuronal and muscular conditions characterised by neuronal hyperactivity in humans and animals (Turin et al., 2023). Tetanus neurotoxin (TeNT), with essentially the opposite muscular effect to BoNT, is yet to be fully realised for its potential medical applications (Conduit et al., 2007; Ovsepian et al., 2019). TeNT is the only known substance that has the potential for localised selective enhancement of motor functions, capable of overcoming flaccid muscles and muscle weakness (Ovsepian et al., 2019). The main reason that TeNT has largely failed to be utilized as a medical intervention in humans and horses is that most will have been vaccinated with tetanus toxoid, resulting in pre-existing passive antibody immunity against tetanus toxin. This has impeded the development of TeNT therapeutic drugs targeting a wide range of neuromuscular conditions (Conduit et al., 2007).

Tetanus toxin increases neuronal tone of muscles by increasing the firing rate of the associated motor neuron. After uptake at the motor neuronal end-plate in the muscle, it is transported retrogradely to motor neuron cell body, then crossing over to the inhibitory interneuron and there reduces synaptic firing by cleaving synaptobrevin, a SNARE protein essential for the release of neurotransmitters. This results in disinhibition and thus an increased neural discharge rate by the motor neuron (Megighian et al., 2021). Therefore, TeNT has the potential to

treat BOAS, by opening or supporting the upper airway by stimulating the upper airway dilator muscles (such as the geniohyoid, levator palatii or stylopharyngeus) through localised, small-dose, TeNT injections (Conduit et al., 2007; Sasse et al., 2005). Improved tone and performance of the geniohyoid may stabilise or prevent caudal collapse of the hyoid and associated structures during respiration, thereby supporting and improving airflow through the upper airway. Proof-of-concept that TeNT could be used to treat Obstructive Sleep Apnoea (OSA), likely associated with BOAS, was first demonstrated in a single case study of a British Bulldog by Sasse et al. (2005). The bulldog, diagnosed with OSA, was successfully treated with TeNT with the number of respiratory events during sleep being significantly reduced. An improvement in daytime breathing was observed lasting for almost six months (data unpublished).

Snoretox-1, is a novel pharmacotherapy solution that combines active tetanus toxin (TeNT) with decoy (inactivated) tetanus toxin. The key development is the inclusion of the antibody decoy, to act as a smokescreen by absorbing any local antibodies resulting from active vaccination or passive environmental exposure, allowing a therapeutic dose of active TeNT to be internalised into the local motor neuron (McLean et al., 2020). Our studies have confirmed that Snoretox-1 is active in a dose-dependent manner in the skeletal muscle of mice that have been vaccinated against tetanus toxin, and has improved effectiveness in increasing muscle tone in vaccinated mice compared to native TeNT alone (McLean et al., 2020).

Study aims and hypotheses

The aim of this study was to apply Snoretox-1 to the rostral geniohyoid of British bulldogs to assess its effectiveness in treating BOAS syndrome clinical signs utilising established BOAS grading systems and owner ratings. We hypothesised that Snoretox-1 injection would result in a clinically significant improvement in BOAS, as indicated by a BOAS assessment accredited veterinarian using The Kennel Club/University of Cambridge's Respiratory Function Grading Scheme and owner subjective ratings of health and wellbeing.

Methodology

Snoretox-1 preparation

The Snoretox-1 formulation comprises a recombinant tetanus toxin (a 1314 amino-acid protein) and a recombinant tetanus toxoid. The toxoid is inactivated through 4 amino acid mutations (E270A, Y374A, R1225E, W1288A) and otherwise is identical to the tetanus toxin. These mutations inactivate the proteolytic activity of the toxoid and reduce binding of the toxoid to the ganglioside, rendering it inactive (McLean et al., 2020). Production and purification of the his-tagged recombinant proteins was similar to McLean et al. (2020). Recombinant proteins were prepared by Treidlia Biovet Pty Ltd (Sydney, Australia).

TeNT Units of activity were determined using an in-house developed *in vivo* mouse bioassay. This assay involves injecting known amounts of TeNT into the gastrocnemius muscle of the hind leg of 10-week-old C57B6 female mice and observing for signs of tetany symptom development. Stage 4 tetany (sustained localised limb tetanus) as defined by Webster and Laurence (1963) was the trial endpoint. The dose required to induce stage 4 tetany at 24 h post-dose was defined as 1 unit in this pilot study. A mouse group size of n = 4 was used. Potency was determined prior to dosing of dogs. A lack of toxin activity for decoy was confirmed by a very high dose in a single mouse at a dose of 12 μg.

Animal trial

All methodology was approved by the RMIT University Animal Ethics Committee (AEC 25887 and AEC 25902), and in accordance with the conditions of Australian Pesticides and Veterinary Medicines Authority (AVPMA: PER7250). Enrolment inclusion/exclusion criteria: British bulldogs of either sex, with owner reported history of BOAS, of age 12 months onwards, accredited veterinarian BOAS rating ≥ 2, anti-tetanus antibody titre $< 100{,}000$ and veterinarian assessed health screening history and check-up including absence of significant illness or gestation. Owner consent was obtained prior to study commencement.

The rostral geniohyoid was selected as the target injection site on the basis of the previous unpublished pilot (dose estimation and tolerance) studies showing potential efficacy, the known airway dilator properties of this muscle and the ease of injection access.

For treatment, dogs were placed under standard sedation and analgesia using medetomidine (Domitor, 10 μg/kg) and butorphanol (Torbugesic, 0.1 mg/kg) for bilateral intramuscular injections of saline (placebo) or Snoretox-1 (treatment) into the left and right geniohyoid muscles (see Fig. 1) in the centre of the geniohyoid rostral compartment, medial to the 406 and 306 teeth. The injection site was then examined by the veterinarian immediately after injection for pain or swelling. Atipamezole (Antisedan) was used as the sedation reversal agent. Standard post-sedation monitoring was then undertaken at the veterinary surgery.

The study was an exploratory single-arm crossover with a sample size of 6. A power calculation was conducted using G*Power (Version 3.1.9.7, Faul et al., 2009). A minimum sample size of 6 was estimated assuming a medium effect size Cohen's $f = 0.6$ (based on previous pilot work), $\alpha = 0.05$ and Power $(1\text{-}\beta) = 0.80$. Initially, dogs received a saline dose using the same sedation and injection site, followed by a post-treatment monitoring period of at least 4 weeks. Subsequently, dogs were dosed with Snoretox-1 at 25 U TeNT/kg followed by a post-treatment monitoring period of at least 12 weeks. Post-treatment monitoring included daily owner monitoring, and veterinary checkups that included BOAS and health assessments at days 14, 28 and then every 4 weeks (±1 week). Post-monitoring periods continued until BOAS grade returned to saline levels. If no improvement in BOAS grade was observed, then after a period of 12 weeks the dog would return for a 50 U TeNT/kg dose of Snoretox-1, followed by the same post-treatment monitoring. The study ended if there was no improvement in BOAS rating to the second dose.

Veterinary BOAS assessment

Veterinary assessments were undertaken prior to dosing and at 14 days (to assess efficacy and check for local toxicity such as spasms or tetany), 28 days, and then every 4 weeks after treatment until the trial endpoints. Trial efficacy endpoints were either no improvement to BOAS

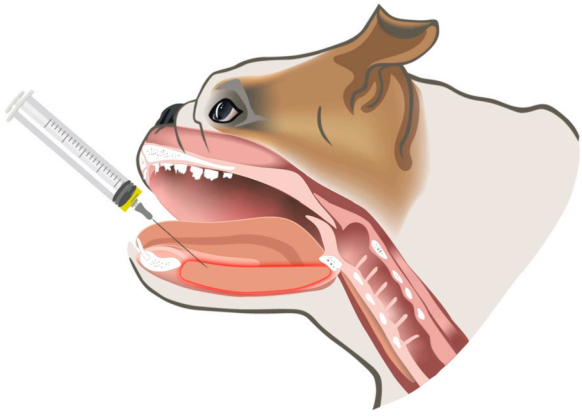

Fig. 1. Dogs were placed under standard sedation and analgesia for bilateral intramuscular injections of Saline or Snoretox-1 (25 U/kg or 50 U/kg) into the rostral geniohyoid muscles (outlined in red). The injection site was then examined by the veterinarian immediately after injection for pain or swelling. Standard post-sedation monitoring was then undertaken at the veterinary surgery.

rating by 12 weeks or a return to pre-treatment BOAS rating following improvement, up to a maximum of 12 months post-treatment. Owner withdrawal from the trial was considered an ethical endpoint. These were undertaken at a veterinary surgery by veterinarians accredited through Dogs Australia as approved assessors to carry out the Cambridge University and Kennel Club UK Respiratory Function Grading Scheme. Standard BOAS assessment procedures were adopted by the veterinarian, as outlined by The Kennel Club/Cambridge BOAS Research Group (Liu et al., 2015). Due to the pilot nature of the study and the fact that the veterinarian administering the treatment was also the assessor, the study was unblinded. This involved respiratory effort assessment before and after a 3-minute trot test where on a lead the dog is encouraged to trot at 4–5 miles per hour by the assessors for 3 min outside in ambient temperatures of 7–22 °C with outdoor humidity ranging between 49% and 87%. BOAS functional level is graded on a 4-point scale. Grade 0 is where a dog is judged as clinically unaffected and free of respiratory signs of BOAS. Grade 1 is when a dog is assessed as clinically unaffected, with mild respiratory signs of BOAS not affecting exercise performance. Grade 2 is when a dog is judged as clinically affected, with clinically relevant respiratory signs. Grade 3 is when a dog is considered clinically affected with severe respiratory signs of BOAS. General health and welfare assessments were also undertaken by the veterinarian at each time point.

Animal owner assessment

After an initial health and BOAS screening undertaken at the veterinary practice, owners received training, including step-by-step instructions by the investigators, on what to look for in their dog regarding tetany symptoms and pain, with reference to monitoring sheets/checklists (See Supplementary Form Owner Monitoring.docx). Throughout the study, owners were required to provide daily ratings to monitor dog health, including alertness, feeding/appetite and drinking behaviours, and observe for any adverse effects including signs of pain or distress as well as signs of tetany. Owner monitoring of sleeping, breathing, activity and stamina was also undertaken to assess owner impression of treatment efficacy. These were recorded on non-validated 5-point scales (e.g. Breathing: "4: much better than before the study", "3: better than before the study", "2: same as before the study", "1: worse than before the study", "0: much worse than before the study").

Anti-tetanus antibody titres

Whole blood samples were collected with a CAT serum clot activator tube at veterinary visits throughout the trial to monitor antibody titres. Samples were allowed to clot at room temperature for 1 h, then spun at 1000 × *g* for 10 min and serum collected. Serum samples were aliquoted and stored at −20 °C until thawed for use.

The decoy-specific antibody titre of each animal was determined in duplicate by ELISA. Briefly, 96 well plates were coated overnight with 5 μg/ml of recombinant decoy in carbonate coating buffer pH 9.6. Plates were washed three times with PBS 0.05% Tween 20 and blocked with PBS 5% skim milk for 1 h at 37 °C. Plates were washed three times with PBS 0.05% Tween 20 then coated with serial dilutions of the dog serum in PBS 1% skim milk, starting at an appropriate concentration for each sample determined by a pilot ELISA. Each plate also had an internal control standard, derived from a dog vaccinated with a registered equine tetanus vaccine (Equivac T, Zoetis). Plates were incubated for 1.5 h at 37 °C then washed three times with PBS 0.05% Tween 20. Wells were coated with 100 μL of Goat anti-dog IgG HRP-conjugated antibodies (A8763 Sigma) diluted to 1:10,000 in PBS 1% skim milk and incubated for 1.5 h at 37 °C. The plates were washed three times with PBS 0.05% Tween 20, developed with TMB chromogenic solution (Invitrogen) in the dark for 10 min, then stopped with 1 M HCl. Absorbance at 450 nm was determined using an iMark plate reader (Bio-Rad). Titres were calculated against internal standards on each plate at a 1:5000 dilution using GraphPad Prism (version 10.5.0).

Statistical analysis

Statistical analysis of the primary outcome (veterinarian BOAS grade) was conducted using the non-parametric Friedman χ^2 test followed by Durbin-Conover post-hoc tests for pairwise comparison. BOAS RFG gradings from 4 weeks after placebo (before Snoretox-1 injection) were considered baseline readings and were analysed against 2 weeks, 4 weeks, 8 weeks and 12 weeks post-treatment.

Results

BOAS ratings at baseline and following saline and Snoretox-1 bilateral geniohyoid injection

Four female and two male British bulldogs (females were 4, 6, 6, and 8 years old, and males were 4 and 8 years old) passed and were included in the study. The dogs were companion animals and remained in domestic care at home with their owners throughout the study, apart from visits to the veterinarian clinic for study procedures. Two of the dogs were previously treated in a pilot study. Accredited veterinarian BOAS grade prior to and following saline and Snoretox-1 bilateral geniohyoid injection are shown in Fig. 2. A reduction in BOAS grade was reported for all dogs. The shortest duration of improvement was 20 weeks post-treatment (excluding Dog 4, which died at 15 weeks, as below), while the longest was dog 1 which at 53 weeks post-treatment (at the trial conclusion) still exhibited improvement in BOAS clinical grades compared to pre-treatment (See Figs. 2 and 3).

Comparison of BOAS gradings before Snoretox-1 injection (4 weeks after placebo) and after Snoretox-1 injection (at 2 weeks, 4 weeks, 8 weeks and 12 weeks post-injection) showed a significant overall difference in ratings pre- and post-treatment (Friedman $\chi^2 = 18.6$, $p < .001$), with BOAS ratings at all time points to 12 weeks after Snoretox-1 treatment significantly lower than prior to Snoretox-1 treatment ($p < .001$).

Videos are available of Dog 1, Dog 3 and Dog 5, both before and after treatment and demonstrate marked improvement of BOAS features (see Supplementary Video Dog 1.mp4, Supplementary Video Dog 3.mp4 and Supplementary Video Dog 5.mp4).

Owner assessment of dog BOAS symptomology

Owner ratings of dog behaviour are shown graphically in Fig. 4. Following treatment, owners rated 3 of the 6 dogs as having improved breathing ('better' or 'much better'), while 2 of the dogs were rated as more active ('more', 'much more') following treatment. A single dog (dog 6) was rated as less active a fortnight after dosing, though returned to normal levels of activity after 8 days. Dog alertness levels were fairly constant throughout the study for all 6 dogs. Owner assessments of dog snoring and sleep quality were mixed; an improvement in snoring was observed after treatment for 2 dogs, while the remaining 4 dogs showed no change or periods of increased snoring after treatment.

Dog weight, TeNT antibody titre, pain and tetany across the study

Fig. 5 shows the dog weight and TeNT antibody titre of the animals across the study protocol up to 24 weeks after Snoretox-1 bilateral geniohyoid injection. Dogs showed slight weight loss following treatment. Increased antibody titres of TeNT were observed in all dogs following treatment, reaching maximum titres 2–4 weeks post-treatment before steadily trending back towards pre-treatment levels.

Health assessment and adverse events

Dog 4 died of a genetic cardiac condition during the treatment period. The death occurred 15 weeks following treatment. Therefore, no BOAS ratings were taken for Dog 4 after 12-week post-injection due to its death. This is also why statistical analyses of the full sample were

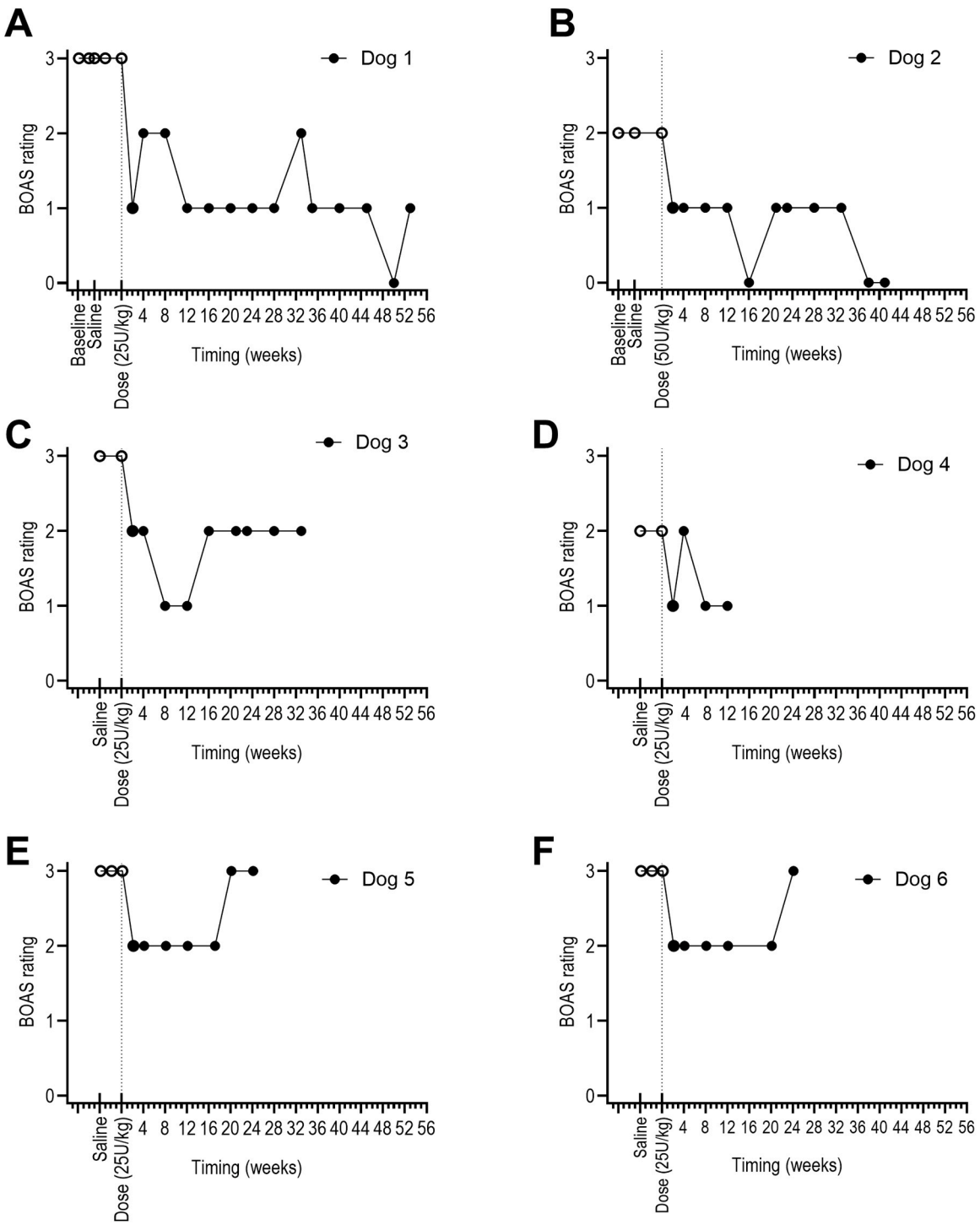

Fig. 2. Accredited Veterinarian BOAS Ratings utilizing The Kennel Club/University of Cambridge Respiratory Function Grading System following Snoretox-1 Bilateral Geniohyoid injection. Ratings listed are taken pre- and post- Saline injection (O), 2 weeks post-treatment then monthly post-treatment (•). Bulldog 2 did not respond to 25U/kg dose so received a second dose (50U/kg) 12 weeks later.

limited to 12 weeks post-injection. Necropsy by an independent veterinary pathologist indicated that the dog died of the fatty form of arrhythmogenic right ventricular cardiomyopathy (ARVC). This is a genetic-based and inherited condition that is a common entity in British bulldogs and Boxers (Cunningham and Dos Santos, 2022; Holdt et al., 2022). The dog was approximately 8.5 years old, which is the median life span of Grade 3 BOAS dogs (O'Neill et al., 2015). The death was considered to be temporally distant from and unrelated to the treatment with Snoretox-1.

Dogs 5 and 6 demonstrated excessive salivation and dysphagia that presented as messy and difficulty eating likely due to inaccurate placement of the injection as discussed below. Symptoms presented approximately 1 week following treatment and lasted up to 5 ½ weeks before complete resolution. Dog 6 also received a wound to its mouth approximately 16 weeks after treatment (while playing with another dog), that resulted in no BOAS assessment at 16 weeks post-injection.

All other dogs maintained good general health and recovery following sedation and injection with placebo or Snoretox-1 treatment.

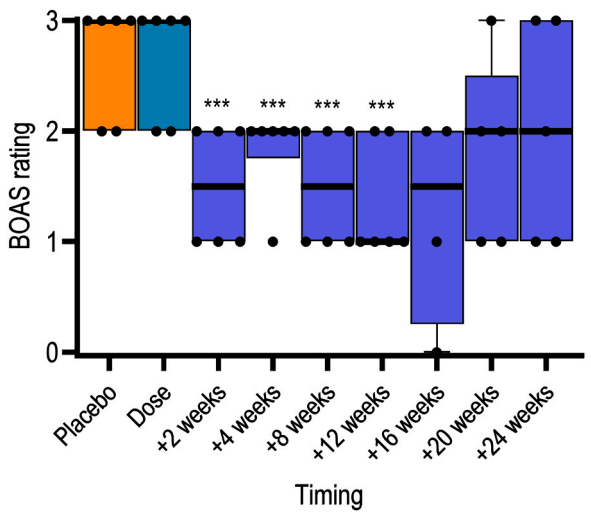

Fig. 3. Combined BOAS ratings utilizing The Kennel Club/University of Cambridge's Respiratory Function Grading System from prior to placebo through to 24 weeks post-treatment. Data are presented as Tukey Box-and-whisker plot (box extending from 25th to 75th percentile with line representing median). Additionally, all individual data points also plotted. Dog dose = 25U/kg, except Dog 2 = 50U/kg. Data for dog 4 collected only to week 12 due to death from genetic cardiac disease (see health assessment and adverse events for details). * Indicates $p < 0.05$, ** Indicates $p < 0.01$, *** Indicates $p < 0.001$.

Discussion

BOAS Syndrome is a highly significant yet often inadequately treated condition. Direct pharmaceutical interventions for the treatment of BOAS in dogs do not currently exist. The period of therapeutic effect of Snoretox-1 was expected to be similar to the therapeutic and cosmetic effect durations observed in treatment with Botulinum toxin (Botox, Dysport, etc) which are reported to be effective for 3–6 months in cosmetic applications and 6–9 weeks in therapeutic applications (Bihari, 2005; Flynn, 2010). However, we observed significantly longer therapeutic effect in 3 of the 6 dogs treated with Snoretox-1. We propose that a reduction of pressure-gradient-related turbulent airflow, resulting in less vibration related barotrauma to upper airway structures such as the soft palate, may result in less oedema and hence more space for airflow. Additionally, such respiratory improvements may lead to better sleep and tolerance for exercise, leading to an overall health improvement for the animal which might explain the maintained reduction in BOAS functional grading beyond 6 months.

Pre-existing immunity to TeNT through vaccination (eg. human, horses) or environmental exposure (eg. dogs) is an impediment to developing potential therapeutic applications. The formulation used in this study contains TeNT and a decoy molecule to overcome this obstacle. A previous study has shown that TeNT itself improved dog breathing while studies in mice have shown that the presence of an excess of decoy molecule can allow TeNT to function in animals with pre-existing immunity (McLean et al., 2020; Sasse et al., 2005). Four of the dogs in this study had not been previously treated with Snoretox-1, while 2 dogs (Dogs 3 and 4) had previously received treatment doses as part of an earlier dose-finding study (data not shown). In addition, dog 2 received the higher dose after not responding to the lower dose 12 weeks beforehand. All dogs responded to treatment, further supporting the presence of a decoy mechanism that allows TeNT to function as a potential therapeutic even after prior exposure and in the presence of pre-existing immunity. These early data represent a vital step towards developing tetanus-based therapeutics, be that for veterinary or human applications in fields such as sleep apnoea.

All dogs treated in this study demonstrated decreases in BOAS functional grading by veterinarians accredited in the use of The Kennel Club/University of Cambridge Respiratory Function Grading Scheme (Liu et al., 2015). This statistically significant decrease in BOAS functional grading (i.e. improvement) was demonstrated at 12 weeks post-injection and was, on average, greater than a one-point drop across all animals on the 4-point scale. On the owner rating scale, some dogs improved whereas others appeared unchanged. When asked about the potential reasons for this lack of change, these owners suggested their dogs preferred a sedentary lifestyle.

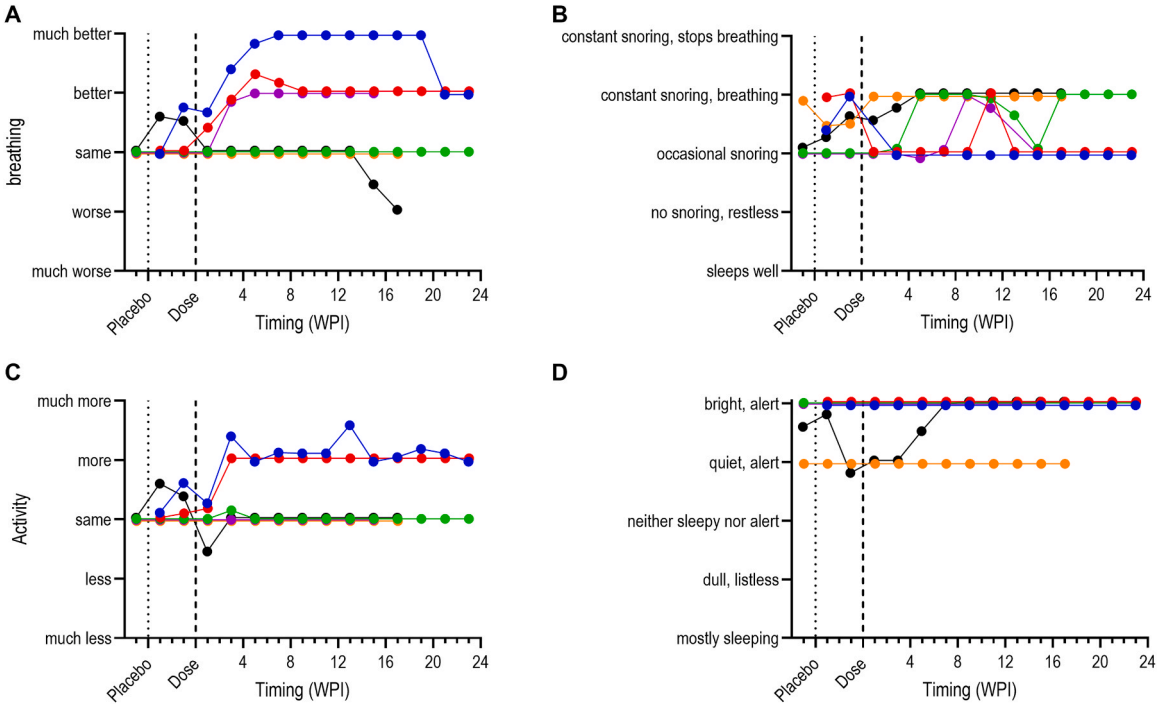

Fig. 4. Owner ratings for A) breathing, B) sleeping, C) activity, and D) alertness. Scores are 2-week average ratings up to 24 weeks post-treatment. Individual data points are slightly offset to improve clarity. WPI -Weeks Post Injection (Snoretox-1).

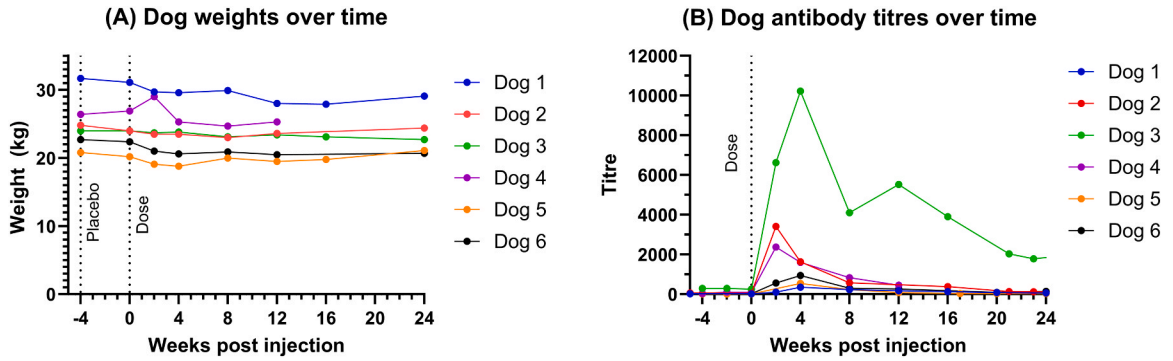

Fig. 5. These graphs show the dog weight (A) and TeNT antibody titre (B) of the animals across the study protocol up to 24 weeks after Snoretox-1 bilateral geniohyoid injection. Increased antibody titres were observed in all dogs following treatment, reaching maximum titres 2–4 weeks post-treatment before steadily trending back towards pre-treatment levels. Dog 3 had previous exposure to TeNT during a dose escalation study which may explain the stronger antibody response.

Two deviations, both related to dosing occurred in this study. Post-treatment potency testing of doses delivered to Dogs 1–4 subsequently indicated that the TeNT units were lower than indicated from pre-treatment testing, considered to be due to an inconsistency in the buffer utilised. This resulted in dogs receiving less than the 25 U TeNT/kg dose. It was estimated they received approximately 0.25 – 0.5 × the TeNT dose indicated (but the correct amount of decoy). Despite this, 3 of the 4 dogs showed improved BOAS ratings, indicating treatment efficacy even at potentially lower doses. The other dog (Dog 2) was retreated after 12 weeks with a 50 U/kg dose, leading to improved BOAS ratings.

When injecting dogs 5 and 6, the syringe needle tip was potentially placed through the geniohyoid and out the other side into the subcutaneous tissue of the chin. It is likely that the full dose was not administered to the target muscle, with a portion of the dose placed into the subcutaneous tissue inferior to the target muscle, namely the chin area. While improvements in breathing (and BOAS ratings) were observed, these dogs subsequently presented with excess salivation and dysphagia, resolving over 5 ½ weeks. These events were recorded as messy eating and/or difficulty eating by owner assessment. A potential explanation for these symptoms is temporary local pharyngeal nerve and/or salivary gland irritation. We did not observe these effects in the other four dogs. The improvements to BOAS ratings observed for these two dogs suggest that Snoretox-1 still acted in the target area, whether slightly offsite or at a reduced potency.

There were mixed results for snoring reported in the owner questionnaires following treatment. As owner ratings are highly subjective, and during the trial period the owner is far more likely to focus on symptoms described in the questionnaire, it is difficult to determine the validity of these observations. One possible explanation for more snoring following treatment is that occasionally improved airflow may result in more soft palate vibration.

This formulation of Snoretox-1 may have application as an adjunct or combination therapy with surgery, or as a first-line of treatment for BOAS in dogs unsuitable for surgery. Further applications include treatment of dogs who have not responded to BOAS surgery adequately, and as a pre-anaesthetic treatment for BOAS dogs who are undergoing major surgery of any type, as a pre-operative dose 4–12 weeks before elective surgery might improve the anaesthetic profile by stabilising the upper airway and reducing oedema pre-anaesthetic. Future research will focus on extending these initial scientific results into a larger cohort and extending into the other BOAS-afflicted breeds such as pugs and French bulldogs. The potential for treatment to increase life expectancy of dogs assessed to suffer from severe BOAS will be investigated.

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This study was funded by Snoretox Ltd and RMIT University. Snoretox Ltd provided the Snoretox-1. Several authors are shareholders and/or employees of Snoretox Ltd. The funder contributed to the study concept and supported the decision to submit the manuscript. Animal procedures were approved by the RMIT University Animal Ethics Committee.

CRediT authorship contribution statement

Russell Conduit: Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **David W. Swift:** Investigation. **Arthur House:** Investigation. **Peter M. Smooker:** Supervision. **Danny Aliano:** Writing – review & editing. **Luke J. Norbury:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Anthony Sasse:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Maurice Newport:** Investigation. **Thomas McLean:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

This study was funded by Snoretox Pty Ltd and RMIT University. Snoretox Pty Ltd provided Snoretox-1. Several authors are shareholders and/or employees of Snoretox Pty Ltd. The funder contributed to the study concept and supported the decision to submit the manuscript.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tvjl.2026.106636.

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Glossary

Brachycephalic Obstructive Airway Syndrome (BOAS): A syndrome of upper-airway obstruction in brachycephalic dogs arising from airway anatomical and functional

abnormalities.

Respiratory Functional Grading Scheme (RFGS): Validated BOAS severity scoring system incorporating exercise-induced respiratory assessment and recovery characteristics.

Snoretox-1: An investigational veterinary biologic comprising a modified tetanus-derived neuromodulatory protein together with a functionally inactive immunogenic decoy molecule, designed to bypass pre-existing passive anti-tetanus antibody mediated immunity and thereby restore the potential of tetanus-based therapeutics to increase localised muscle tone. Under evaluation for improving upper-airway muscle tone in dogs with BOAS, among other indications.