Paper

Dexmedetomidine oromucosal gel for noise-associated acute anxiety and fear in dogs—a randomised, double-blind, placebo-controlled clinical study

M. Korpivaara, K. Laapas, M. Huhtinen, B. Schöning, K. Overall

The aim of this randomised, double-blind, placebo-controlled, clinical-field study was to evaluate the effect of dexmedetomidine oromucosal gel at subsedative doses in alleviation of noise-associated acute anxiety and fear in dogs. On New Year's Eve, 182 dogs with a history of acute anxiety and fear associated with fireworks received treatment as needed up to five times: 89 dogs received dexmedetomidine and 93 dogs received placebo. For the primary efficacy variables, dog owners assessed the overall treatment effect as well as signs and extent of anxiety and fear. The overall treatment effect was statistically significant (P<0.0001). An excellent or good treatment effect was reported for a higher proportion of dogs treated with dexmedetomidine (64/89, 72 per cent) than those receiving placebo (34/93, 37 per cent). Additionally, dexmedetomidine-treated dogs expressed significantly (P<0.0314) fewer signs of fear and anxiety despite the noise of fireworks. No local tolerance or clinical safety concerns occurred during the study. This study demonstrated that oromucosal dexmedetomidine at subsedative doses alleviates noise-associated acute anxiety and fear in dogs.

Introduction

Sensitivity to noise is among the most common behavioural concerns for dog owners but is often inadequately or inefficiently treated (Sherman and Mills 2008, Blackwell and others 2013). Up to 49 per cent of owners report that their dogs show fear responses to some sort of noise, the most common being fearful responses to fireworks (Blackwell and others 2013). Similar responses to fireworks, gunshots and thunder are frequently seen, suggesting that responses to one loud noise are generalisable to others (Overall and others 2001, Blackwell and others 2013). Regular exposure to anxiety-inducing stimuli over a period of time can negatively affect the physical, mental or social health of the dog (Dreschel 2010, Mills and others 2014) and thus reduce the quality of life. Despite the high prevalence of this welfare concern, less than a third of these owners currently seek professional advice (Blackwell and others 2013).

Dexmedetomidine, an α-2 adrenoceptor agonist (α-2 agonist), has been shown to be anxiolytic in laboratory animal models (Millan and others 2000) as well as in human beings (Mantz 2000). Clonidine, another α-2 agonist, was also found efficacious in alleviating acute canine fear-based behaviour problems at subsedative doses (Ogata and Dodman 2011). The anxiolytic and sedative/hypnotic actions of dexmedetomidine are mediated through inhibition of locus coeruleus firing. The locus coeruleus is a pontine nucleus containing one of the highest densities of α-2 adrenoceptors and is a key source of noradrenergic innervation of the forebrain (Barnes and others 1988). The locus coeruleus is an important modulator of sympathetic tone, vigilance and attention. There is abundant evidence to show that overactivation of the noradrenergic neurotransmission (increased release of noradrenaline in the locus coeruleus) induces fear/anxiety in experimental animals exposed to stress (Tanaka and others 2000). In a pilot study conducted on 36 client-owned dogs suffering from acute anxiety due to noise, oromucosal dexmedetomidine gel at subsedative doses of 125 and 250 μg/m^2 (up to five times on New Year's Eve) was shown to be safe and efficacious (Korpivaara and others 2014). This pilot study as well as recent literature (Mariti and others 2012, Blackwell and others 2013, Lakestani and others 2014) demonstrated that dog owners are not only familiar with common behavioural signs of acute anxiety, but are also able to recognise them.

The primary objective of this study was to compare the efficacy of dexmedetomidine 0.1 mg/ml oromucosal gel with placebo gel in alleviation of acute anxiety and fear associated with noise in dogs.

Materials and methods

A total of 17 veterinary clinics in Germany and Finland participated in this randomised, double-blind, placebo-controlled, parallel-group, clinical-field study. The study was conducted between November 2012 and January 2013 and the main study assessments were performed by the dog owners on New Year's Eve 2012.

M. Korpivaara, DVM,
K. Laapas, MSc,
M. Huhtinen, DVM, PhD,
Orion Corporation Orion Pharma, R&D,
Turku, Finland
B. Schöning, DVM, PhD,
Hohensasel 16, Hamburg 22395,
Germany
K. Overall, MA, VMD, PhD, DACVB,

Biology Department, University of
Pennsylvania, Philadelphia, PA 19104,
USA

E-mail for correspondence: mira.
korpivaara@orionpharma.com

Provenance: Not commissioned;
externally peer reviewed.

Accepted January 22, 2017

The study protocol was approved by the regulatory authorities in both countries. Informed consent was obtained from owners before enrolling their dogs in the study. The study was conducted in accordance with the principles of good clinical practice. The welfare, treatment and care of dogs were ensured by veterinary supervision.

Animal selection

Eligible dogs were recruited from the clientele of the participating veterinary clinics and through advertisements in the clinics and social media. Male and female dogs of any breed were eligible to participate in the study if they were at least two years of age, weighed at least 2 kg and had a history of suffering from acute anxiety and fear due to fireworks. Dogs had to also have American Society of Anaesthesiologists (ASA) status I or II (healthy or with mild systemic disease). The owners were required to be able to administer medication, perform all assessments and spend the New Year's Eve in an environment where the dog would be exposed to the noise of fireworks.

Dogs were excluded from participating in the study if they were concurrently treated with psychoactive medications, homeopathic remedies, pheromones, nutraceuticals, special diets or thundershirt for anxiety and fear or had received behavioural training since the previous New Year's Eve. Other reasons for exclusion were the presence of other dogs suffering from anxiety and/or fear associated with noise in the same household, dental or gingival diseases that could have had an effect on absorption of the study treatment, pregnancy or lactation, a history of hypersensitivity against or any other problems with the application of α-2 adrenergic agonists, participation in earlier studies with dexmedetomidine gel or concurrent participation in any other clinical study.

Administration of medications such as sympathomimetic amines, anticholinergics, tranquilisers or sedatives that could have affected the evaluation of the effect of dexmedetomidine was forbidden for two days before the treatment and strongly discouraged during the treatment period. Feeding was not restricted during the study.

Treatments

The eligible dogs were randomly assigned in a 1:1 ratio to receive either 0.1 mg/ml dexmedetomidine oromucosal gel at a dose of 125 μg/m^2 or an equivalent volume of placebo gel applied to oral mucosa as needed up to five times. Randomisation was conducted by an independent randomisation specialist using computer software before the study start. Randomisation was balanced in each veterinary clinic (n=17) in blocks of 4. All owners and study personnel were masked to treatment allocation. A dosage of 0.1 mg/ml of dexmedetomidine oromucosal gel (Orion Corporation, Finland) and placebo were provided in identical prefilled syringes in which the placebo gel was identical in appearance to the dexmedetomidine gel.

Veterinarians prescribed the gel dose for each dog based on the dog's bodyweight. The owners were instructed to start the study treatment either pre-emptively one hour before the anticipated start of fireworks or immediately when the first fireworks were heard or the dog began to show signs of anxiety and fear. The gel was applied on the buccal mucosa without allowing the dog to swallow the medication. Redosing could be performed if the dog began to show signs of anxiety or fear again, but not sooner than two hours after the previous dose to avoid potential cumulative effect of dexmedetomidine. The two-hour interval was chosen based on the pharmacokinetic properties of dexmedetomidine in dogs (Capello and others 2015, Sileo [summary of product characteristics] 2015). After oromucosal administration, the maximum concentration of dexmedetomidine occurs at about 0.6 hours and the elimination half-life is about two hours, ranging from 0.5 to 3 hours.

Assessments

A veterinarian examined the dog and interviewed the dog owner within four weeks of the baseline assessments. The veterinarians were advised not to give any behavioural or management recommendations to dog owners. The owner performed the baseline assessments two days before New Year's Eve, and the effectiveness, safety and product usability assessments on New Year's Eve. The owner visited the clinic or was contacted by the clinic personnel by phone during the first two weeks of January.

Overall treatment effect (compared with previous New Year's Eves without treatment)

The owner assessed the overall effect of study treatment on their dog's behaviour at least two hours after the last dose compared with the dogs' reactions in previous year(s) without treatment using the scale presented in Table 1.

Signs and extent of anxiety and fear

The owner recorded the signs of anxiety and fear and the extent of each sign two days before New Year's Eve, and before and one hour and two hours after each dose on New Year's Eve using the scale presented in Box 1. These well-characterised signs were chosen based on published behavioural studies (Overall and others 2006, Cracknell and Mills 2008, Sherman and Mills 2008). In conjunction with behavioural signs, the owners estimated the intensity of fireworks using the following scores: 1=no fireworks, 2=mild fireworks (distant sounds/lights), 3=moderate fireworks (occasional sounds/lights nearby) and 4=intense fireworks (continuous/loud).

Clinical success of treatment (success/failure)

Clinical success of treatment was analysed as a composite variable to capture the clinical effects of the treatment, that is, alleviation of acute anxiety and fear without clinical sedation in the presence of fear-provoking stimuli. Dogs that had excellent or good treatment effect and showed no or only few transient signs of fear and anxiety and lacked signs of sedation were subsumed in the success group. Dogs that had no or some treatment effect or increased reactions were subsumed in the failure group. In addition, in this conservative interpretation, dogs that had excellent or good treatment effect (Table 1) but presented signs of fear and anxiety (Box 1: score 3 or 4 in at least three behavioural signs) or showed signs associated with sedation during a functional alertness assessment (Table 2: score 4 once or score 3 at least twice) were transferred to the failure group.

Usability of the product

The owners assessed the usability of the product and user friendliness of the dosage form and formulation at least two hours after the last dose using a 4-point scale: 1 (very easy), 2 (quite easy), 3 (somewhat difficult) and 4 (very difficult). The owners also recorded the time of dosing(s) and all potential issues when administering the product.

TABLE 1: Owner assessment of overall treatment effect during the study compared with previous year(s) without treatment

Score	Description of treatment effect*
1	Excellent effect: the dog does not react to fireworks with anxious/fearful behaviour at all.
2	Good effect: the dog's reactions are mild and it can calm down.
3	Some effect: the dog is reacting somewhat less/milder than in previous year(s) but it cannot calm down.
4	No effect: there is no reduction/change in the dog's reactions compared with previous year(s).
5	Worse: the dog's reaction to fireworks is stronger than in previous year(s).

*The assessment was done once at least two hours after the last dose

> **BOX 1: Owner assessment of signs and extent of anxiety and fear: description of behaviour**
>
> ▸ **Panting**
> ▸ **Trembling**
> ▸ **Vocalising:** any kind: whining, barking, growling, howling, etc
> ▸ **Pacing:** frequent change of place/running around, restlessness
> ▸ **Seeking people:** clinging, climbing in lap, pawing at, trying to sit behind or under, following, etc
> ▸ **Trying to hide:** under/behind beds, doors, furniture, dark rooms, etc
> ▸ **Trying to escape**
> ▸ **Freezing:** the absence of movement except for respiration
> ▸ **Refusing to eat food/treats**
> ▸ **Inappropriate urination:** a housetrained dog urinates indoors or does not urinate when outside
> ▸ **Inappropriate defecation:** a housetrained dog defecates indoors
> ▸ **Salivating**
>
> Baseline was established once two days before the anticipated event of fireworks. After that the assessments were carried out before and one hour and two hours after each dosing. For this assessment, the owner observed the dog and assessed the following: "Within the previous 15 minutes my dog has shown the following behaviour". The owners estimated the extent of each behaviour using the following scores: 0=none, 1=only a few times, 2=some of the time, 3=most of the time and 4=continuously

Safety variables

The owners assessed the local tolerance by observing the dog's oral mucosa before and every two hours after each dose. To assess the signs of potential sedative effects of the treatment, the owners assessed functional alertness of the dog using the scale presented in Table 2, modified from Jiménez and others (2012). Functional alertness was assessed two days before New Year's Eve (as close as possible to the anticipated start time of fireworks on the New Year's Eve) and one hour after each dose. Adverse events were recorded spontaneously during the study.

Statistical analyses

Sample size calculation was based on the previous study (Korpivaara and others 2014). A sample size of 65 dogs in each group was calculated to have a 95 per cent power to detect a difference in the five categories of the first co-primary variable with a two-sided test at 5 per cent level. Using an estimated dropout rate of 10 per cent, 70 dogs were to be recruited for each group.

All randomised dogs that received at least one dose of study treatment were included in the statistical analyses.

TABLE 2: Functional alertness assessment, modified from Jiménez and others (2012)

Score	Description
1	The dog is able to stand up and walk normally. The dog is fully responsive.
2	The dog is able to stand up and walk normally but is slow to respond to stimuli.
3	The dog is able to stand up, but reluctant to walk and its movements are uncoordinated. The dog is slow to respond to stimuli.
4	The dog is unable to stand up and walk, unresponsive to stimuli (eg, when being called), drowsy or sleepy.

Following instructions were given to the dog owner: "In order to perform the functional assessment, please go to the other side of the room and call your dog to you. Special attention should be paid on evaluation of the dog's ability to walk"

The overall effect of study treatment was analysed as the first co-primary efficacy variable using a generalised linear model with cumulative logit as a link function to obtain OR and 95 per cent CIs. A sensitivity analysis, excluding dogs that showed signs of potential sedation (score 4 once or score 3 at least twice in functional alertness assessment), was also performed.

The second co-primary variable was the behavioural sum score, assessed one hour after each dose. The behavioural sum scores were calculated by summing the extent of signs (from 0 to 4) from the 12 signs. The total possible sum score for a dog per time point was 48, with lower scores indicating less distress. The mean sum scores, as well as the individual signs, were analysed over time (from first dose to fourth dose) with a repeated measures analyses of covariance, including treatment, time and treatment by time interaction as fixed effects, predose score and firework intensity as covariates and subject as a random effect. Dogs scoring 3 or 4 in functional alertness assessment were excluded from this analysis at that time point. The fifth dose was excluded due to too few observations.

The clinical success was analysed as an additional dichotomised variable with a logistic regression model, including treatment, centre and treatment by centre interaction as covariates. The time between doses was analysed using a normal mixed model with treatment as a fixed effect and the administration frequency with a chi-squared test. Functional alertness was analysed as a dichotomised variable by time point using Fisher's exact test.

All tests were two-sided and a 5 per cent significance level was used in all comparisons.

SAS statistics software V.9.1 (SAS Institute, Cary, North Carolina, USA) was used.

Results
Animals

A total of 188 client-owned dogs were screened for the study, and 187 dogs were randomly allocated to receive either dexmedetomidine (n=91) or placebo (n=96). Five dogs did not receive the treatment: one owner did not give the treatment as they decided to spend the New Year's Eve in an area devoid of fireworks; four owners did not specify why they decided not to give the treatment. One hundred and eighty-two dogs received the treatment: 89 dogs received dexmedetomidine and 93 dogs received placebo. The demographic and baseline characteristics were well balanced with respect to age, sex, weight and neuter status across the treatment groups (Table 3). Twenty-one dogs (7 dogs on dexmedetomidine and 14 dogs on placebo) prematurely discontinued the study, with the most common reason being lack of efficacy. All dogs that received the treatment were exposed to fireworks.

Overall treatment effect (compared with previous New Year's Eves without treatment)

A better overall treatment effect was observed for dogs treated with dexmedetomidine than those on placebo; the OR in favour of dexmedetomidine was 3.4 (95 per cent CI 1.95 to 5.99, P<0.0001) (Table 4, Fig 1). A higher proportion of dogs were reported to have good or excellent treatment effect in the dexmedetomidine group (64/89, 72 per cent) than in the placebo group (34/93, 37 per cent).

To show that the results were due to the anxiolytic effects of the study treatment, a sensitivity analysis excluding dogs showing signs of potential sedation (eight dogs given dexmedetomidine and three dogs given placebo) was performed. The results of the sensitivity analysis concurred with the primary analysis confirming the anxiolytic effect of dexmedetomidine (Table 4). The OR in favour of dexmedetomidine was 3.25 (95 per cent CI 1.84 to 5.74, P<0.0001).

Signs and extent of anxiety and fear

Dogs treated with dexmedetomidine expressed significantly fewer signs of fear and anxiety despite the noise of fireworks.

TABLE 3: Demographic and baseline characteristics

Variable	Dexmedetomidine (n=89)	Placebo (n=93)	Total (n=182)
Sex			
Female	42 (47.2)	47 (50.5)	89 (48.9)
Male	47 (52.8)	46 (49.5)	93 (51.1)
Age (years)			
Mean (sd)	6.4 (3.3)	6.5 (2.9)	6.4 (3.1)
Median (range)	6.0 (2–17)	6.0 (2–14)	6.0 (2–17)
Weight (kg)			
Mean (sd)	22.4 (12.6)	22.3 (11.1)	22.3 (11.8)
Median (range)	20.7 (5–67)	22.3 (4–60)	21.7 (4–67)
Neutered			
Yes	68 (76.4)	68 (73.1)	136 (74.7)
No	21 (23.6)	25 (26.9)	46 (25.3)
Signs in the behaviour history*			
Trembling	85 (46.7)	86 (47.3)	171 (94.0)
Panting	82 (45.1)	84 (46.2)	166 (91.2)
Pacing	82 (45.1)	78 (42.9)	160 (87.9)
Seeking people	72 (39.6)	72 (39.6)	144 (79.1)
Trying to hide	62 (34.1)	69 (37.9)	131 (72.0)
Refusing to eat food/treats	61 (33.7)	58 (32.0)	119 (65.7)
Trying to escape	48 (26.4)	56 (30.8)	104 (57.1)
Vocalising	41 (22.5)	47 (25.8)	88 (48.4)
Salivating	35 (19.2)	37 (20.3)	72 (39.6)
Freezing	22 (12.1)	27 (14.8)	49 (26.9)
Inappropriate urination	10 (5.5)	17 (9.3)	27 (14.8)
Inappropriate defecation	1 (0.5)	4 (2.2)	5 (2.7)

Data are presented as the number of dogs (per cent), unless otherwise stated.
*Signs that dogs had displayed on previous New Years' Eves without treatment; these signs were collected at the screening visit.

The mean behavioural sum scores were numerically lower (lower scores indicate less distress) for dogs in the dexmedetomidine group compared with those given placebo (Table 5). The estimate of treatment difference over time was −2.16 (95 per cent CI −3.87 to −0.45, P=0.0134) in favour of dexmedetomidine. Analysis of the individual one-hour time points showed a statistically significant (P=0.0003) difference between the treatments after the second dosing: the least squares mean (±se) score was 4.0±0.7 for the dexmedetomidine-treated dogs versus 7.4±0.6 for the placebo-treated dogs.

Of the 12 behavioural signs, dogs treated with dexmedetomidine displayed less panting (overall treatment effect, P<0.0001), trembling (P=0.0056), vocalising (P=0.0084), pacing (P=0.0192) and inappropriate urination (P=0.0314) than those on placebo (see online Supplementary 1).

Clinical success of treatment (success/failure)

After classifying dogs as successes and failures, 15 dogs (11 dogs given dexmedetomidine and 4 dogs given placebo) that showed excellent or good treatment effect but presented signs of fear and anxiety or showed signs of potential sedation were transferred to the failure group. The difference between treatments remained

TABLE 4: Overall treatment effect on the behaviour of the dog compared with previous year(s) without treatment

		Dexmedetomidine		Placebo	
Score	Description	Primary analysis (n=89) n (per cent)	Sensitivity analysis* (n=81) n (per cent)	Primary analysis (n=93) n (per cent)	Sensitivity analysis* (n=90) n (per cent)
1	Excellent effect	15 (16.9)	13 (16.0)	9 (9.7)	9 (10.0)
2	Good effect	49 (55.1)	45 (55.6)	25 (26.9)	23 (25.6)
3	Some effect	10 (11.2)	9 (11.1)	18 (19.4)	17 (18.9)
4	No effect	11 (12.4)	10 (12.3)	36 (38.7)	36 (40.0)
5	Worse	4 (4.5)	4 (4.9)	5 (5.4)	5 (5.6)

*Dogs that showed signs of potential sedation in functional alertness were excluded.

in line with the primary analysis of the treatment effect. A higher proportion of dexmedetomidine-treated dogs (53/89, 59.6 per cent) than placebo-treated dogs (30/93, 32.3 per cent) remained in the success group. The OR for dogs given dexmedetomidine being in the success group was 3.09 (95 per cent CI 1.69 to 5.67, P=0.0003).

Timing of doses

There were no statistically significant differences between groups with respect to time between doses or administration frequency, though the mean time between consecutive doses was numerically longer in dexmedetomidine-treated dogs than in placebo-treated dogs. For example, the second dose of dexmedetomidine was given on average at three hours six minutes after the first dose, while placebo was readministered on average at 2 hours 50 minutes.

Usability of the product

The majority of the owners (84.6 per cent, n=154) found the syringe very easy or quite easy to use. Ones that found it somewhat difficult or difficult to use had usually indicated that the dog was not accustomed to be handled as required for the treatment administration.

Local tolerance

No local irritation of oral mucosa was reported. However, transient local paleness of the oral mucosa was more commonly observed in dogs on dexmedetomidine (13.3–16.9 per cent) compared with those on placebo (2.1–6.6 per cent) until two hours after the third dose.

Alertness

Most dogs (>85 per cent) in both treatment groups were fully functional and scored either 1 or 2 (Table 2) throughout the evening and night. No apparent differences were seen between the treatments over the course of repeated administrations: for the first dose P=0.2036; second dose P=0.2373; third dose P=0.3509 and fourth dose P=0.4103 (Fig 2). Seven dogs in the dexmedetomidine group and two dogs in the placebo group scored 4 (unable to stand up and walk when being called, unresponsive to stimuli, drowsy or sleepy) in the functional alertness assessment.

Adverse events

There were few adverse events reported during the study and none were serious. The most common adverse event was emesis (five events in four dogs given dexmedetomidine and one event in one dog given placebo). A total of nine events were reported for seven dogs given dexmedetomidine and one event for one dog given placebo (Table 6). None of the events resulted in treatment discontinuation.

Discussion

In the presence of anxiety or fear-provoking stimuli, owners desire alleviation of the signs of fear, anxiety and suffering, but at the same time also wish their dogs to be fully functional. Accordingly, the goal of such treatment is anxiety relief without sedation and impairment of functional alertness. Based on the pharmacokinetics, it is not expected that the selected low dose would sedate the dogs as the mean bioavailability with oromucosal dosing has been reported to be 28 per cent (Capello and others 2015, Sileo [summary of product characteristics] 2015).

In this clinical-field study, simple owner-reported scales were used to compare the overall treatment effect, and the presence and intensity of specified behaviours before and after each dose. These scales were chosen because owners are aware of both the situations that induce fear and the specific behavioural pattern their dog displays (Mariti and others 2012, Blackwell and others 2013, Lakestani and others 2014). To keep the circumstances as authentic as possible, the assessments were made when the dogs were exposed to fireworks in their home environment, allowing
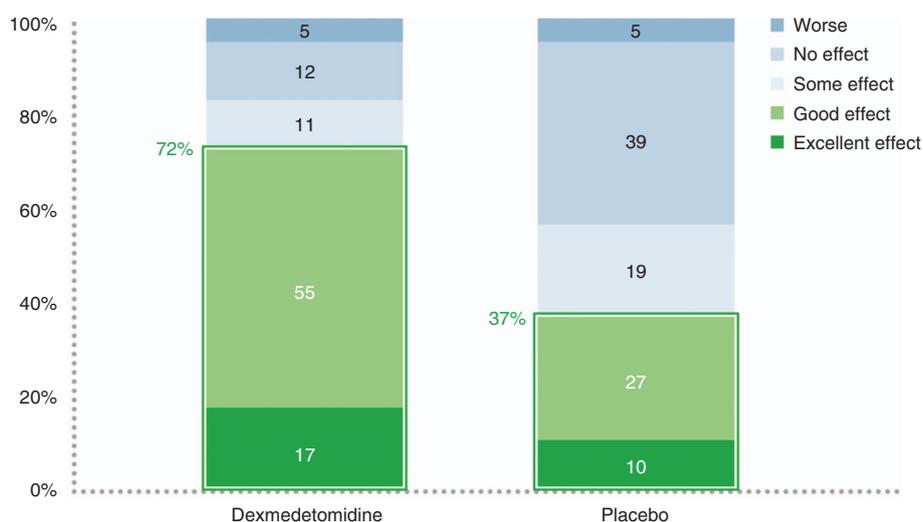
FIG 1: Overall treatment effect on the behaviour of the dog compared with previous year(s) without treatment. The OR in favour of dexmedetomidine was 3.4 with a 95 per cent CI of 1.95 to 5.99, P<0.0001

TABLE 5: Behavioural sum scores by time point

Time point	Dexmedetomidine (n=89) Mean (n)	Dexmedetomidine (n=89) Change from predose	Placebo (n=93) Mean (n)	Placebo (n=93) Change from predose
Screening (previous New Year's Eves)	18.7 (89)		19.1 (93)	
Baseline (2 days before New Year's Eve)	0.8 (89)		1.3 (93)	
First dose				
Predose	4.9 (89)		5.1 (93)	
One-hour postdose	3.8 (89)	−1.1	4.7 (93)	−0.5
Second dose				
Predose	9.1 (75)		10.0 (78)	
One-hour postdose	4.5 (74)	−4.7	8.8 (78)	−1.2
Third dose				
Predose	9.8 (49)		13.3 (51)	
One -hour postdose	6.3 (48)	−3.6	10.0 (50)	−3.1
Fourth dose				
Predose	7.5 (17)		12.0 (24)	
One-hour postdose	6.2 (16)	−1.4	12.0 (23)	−0.6

TABLE 6: Adverse events

Adverse event	Dexmedetomidine (n=89) n (per cent) [n]†	Placebo (n=93) n (per cent) [n]†	P value*
Any events	7 (7.9) [9]	1 (1.1) [1]	8 (4.4) [10]
Emesis	4 (4.5) [5]‡§	1 (1.1) [1]	0.204
Gastroenteritis	1 (1.1) [1]	0	0.489
Periorbital oedema	1 (1.1) [1]§	0	0.489
Drowsiness	1 (1.1) [1]	0	0.489
Sedation	1 (1.1) [1]	0	0.489

*Fisher's exact test was used to compare the number of dogs.
†Data are presented as the number of dogs (per cent) [number of events].
‡One dog experienced emesis twice.
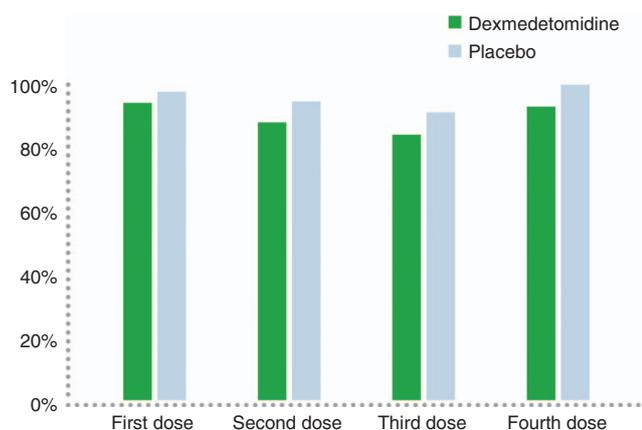§One dog experienced emesis and periorbital oedema.

FIG 2: Proportion of fully functional dogs (scoring either 1 or 2 in functional alertness assessment) by time point. There were no statistically significant differences between treatments

the dog to be fed and handled as usual. This real-life approach was considered to be the most predictive in assessing treatment effect. Although owner-reported scales are a well-accepted method of documenting dog behaviour, a video assessment would have been a more objective method for observation. Video assessments are easy to manage in laboratory settings, but become technically very complex when multiple home environments in various geographic areas are used as study sites, especially during the evening and night. For these practical reasons, video recording was not attempted in this study.

The effect of recall bias, requiring consistent presentation of at least two signs of fear and anxiety at previous fireworks exposures as an inclusion criterion, was accounted for with the prospective, randomised, double-blind and controlled study design. Furthermore, recent research (Tiira and Lohi 2014) based on the same questionnaire for noise-related anxiety (Overall and others 2006) found both an excellent correlation between the questionnaire data and behavioural test as well as a good retest reliability of the questionnaire for specific behavioural signs after a prolonged time (up to eight months).

To ensure validity of the study results, a co-primary strategy was chosen for this study. A significantly positive overall treatment effect (P<0.0001) and a significantly lower level of signs of fear and anxiety (P=0.0134) were both required a priori for the study to be successful. The positive result of the clinical success of the treatment variable also underlined the anxiolytic effect of the treatment: as a dog in the success group (excellent or good effect) per definition did not react fearfully and/or anxiously during the fireworks (excellent effect) or showed only mild and transient reactions but was able to calm down (good effect) despite exposure to fireworks noise. Furthermore, a dog could not belong to the success group if it showed multiple signs of fear and anxiety to high extent (i.e. constantly or most of the time) at any assessment point. At the same time, in order to

show that the positive result was not due to sedation, dogs with the positive result in the overall treatment effect assessment, but scoring 4 (drowsy or sleepy) or multiple scores of 3 (slow to respond to stimuli) in functional alertness assessment were regarded as failures. In this analysis, a dog given dexmedetomidine was three times more likely to be in the success group, as compared with a dog given placebo. Dexmedetomidine-treated dogs displayed less panting trembling, vocalising, pacing and inappropriate urination. This finding suggests that anxiety-associated sympathetic arousal caused by noradrenaline, manifesting in fear and anxiety behaviours, was successfully counteracted by the study treatment in most cases. These results confirm that dogs benefit clinically from this treatment, and that its effect is assessable by the owners. It is also noteworthy that dogs already showing signs of fear and anxiety benefitted from dexmedetomidine gel treatment. This was evident as redosing of the study medication was only permitted when the dog began to show signs of fear and anxiety again.

The response rate of dogs for the placebo is in agreement with other veterinary clinical studies where a caregiver placebo effect is described as a common phenomenon (Muñana and others 2010, Conzemius and Evans 2012). In clinical studies, the expectation of a response can influence subjective interpretation. However, behavioural complaints are often dependent on owner perception (i.e. defined subjectively). The expectation of a response, giving a treatment to an animal, as well as assessment-related and documentation-related activities probably influenced the behaviour of the dog owners (Overall and others 2006, Cracknell and Mills 2008). If owners are more relaxed and less concerned about their dog's behaviour during the fireworks, their behaviour may, in turn, have aided their dogs to behave more calmly.

The functional alertness of the dog was assessed at the expected peak effect of treatment (about one-hour postdose) by calling the dog and paying attention especially to the dog's ability to walk. This assessment was used because dog owners are able to reliably recognise ataxia and reduced responsiveness, which in this study were considered to represent early sedative effects.

At baseline, in the evening two days before the New Year's Eve, 94.5 per cent of the non-treated dogs were fully responsive (score 1). One hour after receiving the first treatment, the percentage of fully responsive dogs dropped similarly in both groups. There were only few dogs in both treatment groups per assessment that scored either 3 or 4. Owners commented that dogs, in both the placebo and dexmedetomidine groups, became less responsive to the owner and owner requests, in general, as night progressed, suggesting that dogs in both groups became fatigued as night progressed and repeated assessments continued.

Both the high prevalence of and the unmet need for treatment of noise-related anxiety and fear became evident when this study was initiated. Enrolment exceeded the recruitment target by 34 per cent within the 4-week recruitment period, requiring that recruitment be actively terminated. This recruitment experience showed that dog owners are increasingly aware of this welfare problem and are willing to use available treatment options.

In conclusion, the anxiolytic properties of dexmedetomidine gel in treating dogs suffering from acute anxiety and fear associated with fireworks were measurable, clinically relevant and statistically significant. Dexmedetomidine significantly reduced behaviours related to fear and anxiety over time, and the overall effect and success of treatment were found superior to placebo. Furthermore, the dose used was safe and devoid of any significant clinical sedative effect. Finally, the novel administration form and delivery system was found by owners to be easy to use across the range of dogs tested.

References
BARNES, K. L., CHERNICKY, C. L., BLOCK, C. H. & FERRARIO, C. M. (1988) Distribution of catecholaminergic neuronal systems in the canine medulla oblongata and pons. *The Journal of Comparative Neurology* **274**, 127–141

BLACKWELL, E. J., BRADSHAW, J. W. S. & CASEY, R. A. (2013) Fear responses to noises in domestic dogs: Prevalence, risk factors and co-occurrence with other fear related behaviour. *Applied Animal Behaviour Science* **145**, 15–25

CAPELLO, A., WENKER, M. A. M., VUORILEHTO, L., EMMEN, H. H. & SALONEN, J. S. (2015) The effect of the blood sampling site on PK parameters after oromucosal administration in dogs. *Poster session presented at the 13th European ISSX Meeting of the International Society for the Study of Xenobiotics*, 22–25 June 2015, Glasgow, UK

CONZEMIUS, M. G. & EVANS, R. B. (2012) Caregiver placebo effect for dogs with lameness from osteoarthritis. *Journal of the American Veterinary Medical Association* **241**, 1314–1319

CRACKNELL, N. R. & MILLS, D. S. (2008) A double-blind placebo-controlled study into the efficacy of a homeopathic remedy for fear of firework noises in the dog (Canis familiaris). *The Veterinary Journal* **177**, 80–88

DRESCHEL, N. A. (2010) The effects of fear and anxiety on health and lifespan in pet dogs. *Applied Animal Behaviour Science* **125**, 157–162

JIMÉNEZ, C. P., MATHIS, A., MORA, S. S., BRODBELT, D. & ALIBHAI, H. (2012) Evaluation of the quality of the recovery after administration of propofol or alfaxalone for induction of anaesthesia in dogs anaesthetized for magnetic resonance imaging. *Veterinary Anaesthesia and Analgesia* **39**, 151–159

KORPIVAARA, M., ASPEGREN, J. & HUHTINEN, M. (2014) Effect of dexmedetomidine oromucosal gel for alleviation of canine acute fear and anxiety associated with noise at sub-sedative doses – A pilot study. *Poster session presented at BSAVA*, 3–6 April 2014, Birmingham, UK

LAKESTANI, N. N., DONALDSON, M. & WARAN, N. (2014) Interpretation of dog behaviour by children and young adults. *Anthrozoos* **27**, 65–80

MANTZ, J. (2000) Alpha2-adrenoceptor agonists: analgesia, sedation, anxiolysis, haemodynamics, respiratory function and weaning. *Best Practice & Research Clinical Anaesthesiology* **14**, 433–448

MARITI, C., GAZZANO, A., MOORE, J. L., BARAGLI, P., CHELLI, L. & SIGHIERI, C. (2012) Perception of dogs' stress by their owners. *Journal of Veterinary Behavior: Clinical Applications and Research* **7**, 213–219

MILLAN, M. J., LEJEUNE, F., GOBERT, A., BROCCO, M., AUCLAIR, A., BOSC, C., RIVET, J. M., LACOSTE, J. M., CORDI, A. & DEKEYNE, A. (2000) S18616, a highly potent spiroimidazoline agonist at alpha(2)-adrenoceptors: II. Influence on monoaminergic transmission, motor function, and anxiety in comparison with dexmedetomidine and clonidine. *Journal of Pharmacology and Experimental Therapeutics* **295**, 1206–1222

MILLS, D., KARAGIANNIS, C. & ZULCH, H. (2014) Stress—its effects on health and behavior: a guide for practitioners. *Veterinary Clinics of North America. Small Animal Practice* **44**, 525–541

MUÑANA, K. R., ZHANG, D. & PATTERSON, E. E. (2010) Placebo effect in canine epilepsy trials. *Journal of Veterinary Internal Medicine* **24**, 166–170

OGATA, N. & DODMAN, N. H. (2011) The use of clonidine in the treatment of fear-based behavior problems in dogs: an open trial. *Journal of Veterinary Behavior: Clinical Applications and Research* **6**, 130–137

OVERALL, K. L., DUNHAM, A. E. & FRANK, D. (2001) Frequency of nonspecific clinical signs in dogs with separation anxiety, thunderstorm phobia, and noise phobia, alone or in combination. *Journal of the American Veterinary Medical Association* **219**, 467–473

OVERALL, K. L., HAMILTON, S. P. & CHANG, M. L. (2006) Understanding the genetic basis of canine anxiety: phenotyping dogs for behavioral, neurochemical,

and genetic assessment. *Journal of Veterinary Behavior: Clinical Applications and Research* **1**, 124–141

SHERMAN, B. L. & MILLS, D. S. (2008) Canine anxieties and phobias: an update on separation anxiety and noise aversions. *Veterinary Clinics of North America: Small Animal Practice* **38**, 1081–1106

SILEO (dexmedetomidine 0.1 mg/ml oromucosal gel for dogs) [summary of product characteristics] Orion Corporation (2015) [last updated August 4, 2016, cited January 3, 2017]. Retrieved from http://www.ema.europa.eu

TANAKA, M., YOSHIDA, M., EMOTO, H. & ISHII, H. (2000) Noradrenaline systems in the hypothalamus, amygdala and locus coeruleus are involved in the provocation of anxiety: basic studies. *European Journal of Pharmacology* **405**, 397–406

TIIRA, K. & LOHI, H. (2014) Reliability and validity of a questionnaire survey in canine anxiety research. *Applied Animal Behaviour Science* **155**, 82–92

CrossMark