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**Research article**

## Homeopathic preparations and separation anxiety in dogs: a pilot study

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### Abstract

Separation Anxiety (SA) is a behavioral syndrome that may affect dogs of different ages and that is characterized by intense clinical signs. Traditional veterinary clinic efforts rely on harmful side effect drugs. Overall, homeopathy handles individual idiosyncrasies and susceptibilities and deal with them using a single medicine through the law of similarity. This study aimed to determine whether individualized homeopathic medicines have a greater effect than placebo for dogs suffering from SA or not, assessing its relation to behavioral settings, cortisol levels, and blood cells count before and after therapy. Owners filled out a score questionnaire. Twenty-one dogs were recruited and repertorized in accordance to classical homeopathy. A pharmacist was responsible to randomize and dispense verum medicine or placebo. On the 30<sup>th</sup> day, reappraisal of owners were allowed altering the dispensed medicine. The final assessment occurred on the 60<sup>th</sup> day. In verum group, destructive behavior analysis had a significant statistical difference intra-group over the trial compared to the placebo group. The mean of cortisol levels in the placebo group was significantly higher on the 60th day of the trial when compared to the verum group, whose levels were sustained over the same period. Although evidenced behavioral improvements could be related to homeopathic preparations, it was not feasible to set any connection between homeopathic interventions, behavioral issues, and plasma components.

**Keywords:** Animal Welfare, Hyper-Attachment, Ultra-dilution

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## INTRODUCTION

The search for a solid relationship has strengthened the bond between men and dogs over the last years (White et al., 2017). This closeness is broadly believed to promote shared contentment, and a dog's personality has a considerable influence on the welfare of both (Tiira and Lohi, 2015). Most puppies are acquired at a neural and socially-sensitive period (6 to 13 weeks old), requiring an intimate relationship. Since social species need to group and keep members together for their safety (Konok et al., 2011), there is a high chance of specific individuals for developing hyper-attachment (Appleby and Pluijmakers, 2003). Domestic dogs can receive high levels of human care, (Zapata et al., 2016) turning socialization with conspecifics into a daily challenging task (Kerepesi et al., 2015). Early life experiences are decisive in predicting fear and anxiety issues (Tiira and Lohi, 2015). In adult dogs (Nicks and Vandenheede, 2014), fear-related conditions like noise phobia (Landsberg et al., 2015), thunderstorm phobia (Overall et al., 2001) and separation anxiety can usually overlap (Storengen et al., 2014).

Separation anxiety (SA) in dogs is a behavioral syndrome characterized by emotional and physiological responses that may vary in intensity and clinical signs (Sherman and Mills, 2008). The most usual are over vocalization, destructive behavior, and inappropriate elimination (urine and feces) when of the owner's physical absence. Dogs may also present nonspecific signs linked to the sympathetic nervous system, such as drooling, emesis, diarrhea, tachycardia, tachypnea, and increased motor function (pacing, trembling, hyperactivity) just before or close to the owner's departure (Takeuchi et al., 2000; Schwartz, 2003).

The leading causes of SA (Storengen et al., 2014; Sherman and Mills, 2008; Palestini et al., 2010) are lack of stimulation, early separation, changes in routine, and traumatic memories of when home alone. Some authors define hyper-attachment as a mandatory requirement for SA (Parthasarathy and Crowell-Davis, 2006) suggesting the degree of distress and the intensity of clinical signs are proportional to the human-dog bond (Palestrini et al., 2010). Classical anthropomorphic explanations refer to it as a bad habit of "trying to keep the owner's attention" by being naughty and spoiled (McCrave, 1991; Sherman, 2008) which commonly result in abandonment or even euthanasia (Cottam et al., 2008).

Methods of stress assessment in dogs can reveal coping strategies, as well as poor welfare (Schöberl et al., 2016). Among these methods, cortisol still stands out as a standard parameter (Zapata et al., 2016). Clinical diagnosis of SA may be distinct from other pathologies, mainly in old dogs (Schwartz, 2003), since emotional and cognitive conditions can also change due to physical illnesses. Qualitative and quantitative information obtained from questionnaires might be useful, although currently there is no applicable standard model (Appleby and Pluijmakers, 2004).

A range of medicines can be listed for the pharmacological treatment of SA in dogs. Conditioning behavior and training (Sherman and Mills, 2008) can be encouraged. Nonetheless, prescriptions should always consider avoiding harmful side effects, particularly in old dogs, and complementary medicine must be considered. High-diluted medicines handle behavioral disturbances by

subtly approaching the organism (behavioral and physical) and considering particular idiosyncrasies (Hahnemann, 2007) towards animal welfare.

This double-blind placebo-controlled pilot study aims to test whether individualized high-diluted preparations might have a more significant effect than the placebo in dogs suffering from SA. Moreover, this study aims to investigate the relationship between treatments, cortisol levels, and leukocyte counts in the blood before and after interventions.

## MATERIALS AND METHODS

### Ethical Approval

This study was approved by the Ethics Committee of Animal Research (protocol number 08/2016—Supplementary files), University of Santo Amaro (UNISA), São Paulo, Brazil, in accordance with the current legislation for Veterinary Research (Brazilian National Council for the Control of Animal Experimentation). It was conducted in a veterinary clinic from July 2016 to June 2017 under the supervision of a homeopathic veterinary surgeon. Participation was voluntary, and all procedures were allowed through an informed consent form signed by the owners of all dogs.

### Subjects

Recruitment occurred via social media advertisements, and the final selection consisted of twenty-one dogs. In their first visit, the veterinary doctor conveyed all necessary information to the owners whose dogs had met all initial inclusion criteria (Table 1). The owners also completed the first questionnaire to provide further information about the subjects (habits and routine). Dogs had to present the three main signs for SA (destructive behavior, over vocalization, and abnormal elimination of feces or urine) regardless of their score (main questions in Table 2).

**Table 1** Inclusion criteria (based in Cracknell and Mills, 2008; Blackwell et al., 2015).

Dogs were over 1 year of age (no outlined age limit).
Dogs should be home alone for a period during the day.
Dogs should not have medical conditions.
Dogs should not be receiving concurrent pharmacological treatment.
Dogs should not be aggressive.

**Table 2** Score level (based in Temesi et al., 2014).

1 – Does your dog present a destructive behavior when you are about to leave the house or when you do so?
2 – Does your dog present over vocalization (barking, weeping, howling) when you are about to leave the house or when you do so?
3 – Does your dog present an inappropriate elimination of feces and/or urine when you are about to leave the house or when you do so?
1 – seldom
2 – sometimes
3 – usually
4 – always

## High-diluted Preparations

On the first day of the trial, the owner's account on the appointment with the homeopathic veterinary surgeon enabled the analysis of dogs' reactions when their owners were about to leave, upon their return and how the dogs behaved during this lonely period. Along with these behaviors, the assessment of life history (including diseases), specific personality traits, temperament, desires, usual conditions, and facing different situations (walking around, confronting strangers and other dogs, relation to conspecifics) elicited symptoms transferred to the homeopathic language (Table 3). A Brazilian repertory guided this process (Ribeiro Filho, 2014). Finally, each dog received an individual prescription of a high-diluted preparation in the 30C-potentization.

**Table 3** Behaviour converted into repertorial language

Clinical signs	Repertorial language
Fear of sudden and loud noise	- Hypersensitivity to noise
Fear of rain, thunder, and thunderstorms	- Thunderstorm phobia
Excessive restlessness and pacing when left or about to be left on its own	- Foretaste disturbance - Fear of being alone - Worsened when alone
Scratches doors, windows, floor with trembling and vocalization	- Panic disorder
Excessive greetings when owner returns	- Excitation disturbance
Presence of feces and urine in abnormal places when owner returns	- Fear of being alone - Revengeful, rancorous
Following the main tutor or other members	- Wish of company - Lack of self-confidence
Following the main tutor, the whole time	- Hyper-attachment
Furniture destruction, self-mutilation	- Destructive behavior
Barking, whining, crying, howling	- Over vocalization
Loss of appetite, thirsty and no excrements when owner returns	- Sadness, depression
The dog stays by the front door until owner arrives	- Passionate
The dog behaves fearfully towards other dogs	- Misanthropy
The dog avoids other dogs	- Fear
The dog behaves submissively when facing other dogs	- Lack of self-confidence
The dog acts fearfully when unfamiliar persons visit your home	- Aversion to foreign people
The dog behaves fearfully towards unfamiliar people	

## Plasma investigations

Blood samples were collected in the first appointment, during the morning, not to tamper with the subjects' circadian rhythm and after a 12-hour fasting period. A commercial chemiluminescent enzyme immunoassay kit was applied to measure canine cortisol blood concentrations, containing polyclonal rabbit anti-cortisol antibodies with a 0,2 µg/dl (5,5 nmol/L) analytical sensitivity. Despite the proneness for inter-assay variability with low values in some analyzers, no significant difference was attested when comparing chemiluminescence to more expensive radioimmunoassay techniques (Russel et al., 2007). Total leukocytes, lymphocytes, and neutrophils count were also verified.

## Blinding Process and Randomization

A homeopathic pharmacy handled the prescriptions and dispensed the verum high-diluted preparations (a potentized homeopathic medicine) and the placebo (control solution with the same 30% hydroalcoholic solution as the verum medicine) to owners. A greater verum group was designed, in a 2:1 ratio to the placebo group, in an attempt to highlight behavioral improvement findings.

All preparations were freshly hand-made, and vials were labeled with the name of the medication. The pharmacist randomly provided a vial to each owner and noted the respective code on a specific table. Then, the owners were instructed to shake the vials and drip five drops into their dog's mouth every morning, 30 minutes before their meal, for 30 days. The identity corresponding to the codes remained hidden in a sealed envelope, held by the pharmacist until the end of the statistics processing.

## Behavioral Analysis – Owners and Veterinary Assessment

The first assessment took place in a follow-up consultation (30<sup>th</sup> day), with the owners answering the same questionnaire to evaluate the behavioral progress of subjects. The owner's rating were the basis for the information analysis, and special attention was given to the three main behaviors related to the syndrome. The veterinary assessment was based on the doctor's perception of welfare (good sleeping, feeding, and disposition) and clinical examination of dogs. The treatment was maintained as the standard protocol for behavioral improvements. A new prescription was applied with a different potentization for dogs with no alterations, varying from a new daily preparation on the 30 C or even a single dose on the 200 C. The pharmacist performed the same protocol, keeping the dogs in their original group (verum or placebo) for another thirty-day treatment period (Table 4).

**Table 4** Groups and interventions

Dog	Groups		High dilution medicines	
	Verum	Placebo	Day 0	Day 30
1	X		Lycopodium clavatum 30 cH	Lycopodium clavatum 30 cH
2		X	Lycopodium clavatum 30 cH	Lycopodium clavatum 200 cH
3	X		Ignatia amara 30 cH	Ignatia amara 200 cH
4		X	Arsenicum album 30 cH	Lachesis 30 cH
5	X		Phosphorus 30 cH	Phosphorus 200 cH
6	X		Arsenicum album 30 cH	Arsenicum album 30 cH
7		X	Lycopodium clavatum 30 cH	Phosphorus 30 cH
8		X	Ignatia amara 30 cH	Ignatia amara 200 cH
9	X		Arsenicum album 30 cH	Arsenicum album 30 cH
10	X		Calcarea carbonica 30 cH	Calcarea carbonica 30 cH
11	X		Argentum nitricum 30 cH	Argentum nitricum 200 cH
12		X	Lachesis 30 cH	Lachesis 200 cH
13		X	Lachesis 30 cH	Lachesis 200 cH
14		X	Ignatia amara 30 cH	Ignatia amara 200 cH
15	X		Pulsatilla nigricans 30 cH	Pulsatilla nigricans 200 cH
16	X		Natrum muriaticum 30 cH	Natrum muriaticum 200 cH
17	X		Nux-vomica 30 cH	Nux-vomica 30 cH
18	X		Arsenicum album 30 cH	Arsenicum album 200 cH
19	X		Phosphorus 30 cH	Phosphorus 200 cH
20	X		Nux-vomica 30 cH	Nux-vomica 200 cH
21	X		Natrum muriaticum 30 cH	Natrum muriaticum 200 cH

## Outcome

On the 60<sup>th</sup> day of the trial, owners responded to the last questionnaire, and this final assessment was considered the primary behavioral outcome. In addition, new blood samples were collected for comparisons. At the end of statistical analysis, the groups formed were: 14 dogs in the verum group and 7 dogs in the placebo group. Although animal welfare was the priority, all dogs were monitored for 60 days whether or not they had completed the trial. Any deviations from the original protocol or significant changes in the dog's environment that might interfere with the results were considered as exclusion criteria.

## Statistical Analysis

Statistical analysis was performed using Statistica version 12.0 (StatSoft, 2014) or SPSS Statistics version 21 (IBM, 2012) for Windows. Graphs were performed with GraphPad Prism version 6.0 (GraphPad, 2012) for Windows. Plasmatic investigations (cortisol levels and total leukocytes, neutrophils, and lymphocytes) were evaluated through a two-way mixed ANOVA. There were no outliers, as assessed by box plot investigation. Data were normally distributed, as assessed by Shapiro-Wilk's test of normality ( $p > .05$ ) and by inspection of quartile–quartile plots (Q–Q plots). There was



homogeneity of variances ( $p > .05$ ) as assessed by Levene's test. To indicate how strong is the proportion of the total variance in a dependent variable that is associated with the membership of different groups defined by the independent variable, it is a good habit to also report the so-called effect size. The partial eta-squared (partial  $\eta^2$ ) and Cohen's  $d$ , therefore, have been reported (Cohen, 1988; Espírito-Santo and Daniel, 2018). Results are presented as the mean (SD) standard deviation unless otherwise stated, and the significance level was set at 0.05. Due to the ordinal-level information of the behavioral assessment variables, non-parametric analysis was chosen (Field, 2013; Corty, 2016; Laerd Statistics, 2017). A Kruskal-Wallis test was run to determine if there were differences in destructive behavior, over vocalization, and abnormal elimination of feces and/or urine in time, during a 60-days follow-up program. Pairwise comparisons were performed using Dunn's procedure (Dunn, 1964) with a Bonferroni correction for multiple comparisons (statistical significance was accepted at the  $p < .0033$  level). The eta-squared ( $\eta^2$ ) and Cohen's  $d$  (Cohen, 1988; Tomczak and Tomczak, 2014) were also computed for the Kruskal-Wallis test. However, since there is not a single agreed method of calculating effect size for non-parametric tests and as the aforementioned measures are more biased than the epsilon-squared ( $\epsilon^2$ ), it has also been reported. A commonly used interpretation for effect sizes is to refer them as *small* (partial  $\eta^2 = .01$ ;  $\eta^2 = .02$ ;  $d = .2$ ;  $\epsilon^2 = .04$ ), *medium* (partial  $\eta^2 = .06$ ;  $\eta^2 = .13$ ;  $d = .5$ ;  $\epsilon^2 = .25$ ), and *large* (partial  $\eta^2 = 0.14$ ;  $\eta^2 = .26$ ;  $d = 0.8$ ;  $\epsilon^2 = .64$ ) based on benchmarks suggested by Cohen (1988) and Ferguson (2009).

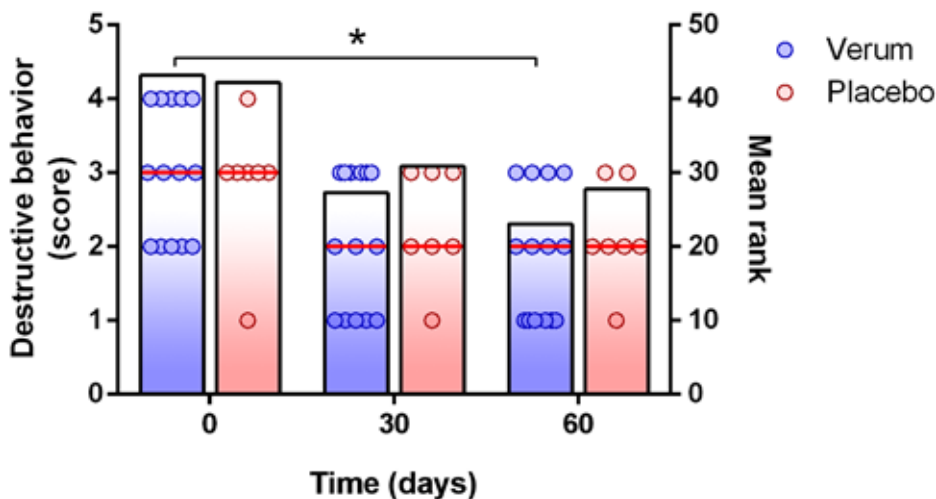
## RESULTS

### The Effect of Time and Treatments

#### Behavioral assessment

Due to the ordinal-level information of the behavioral assessment variables, non-parametric analysis was chosen (Field, 2013; Corty, 2016; Laerd Statistics, 2017). The lack of data normality on behavioral assessment, as assessed by Shapiro-Wilk's test of normality ( $p < .05$ ), suggested the adoption of non-parametric models and, consequently, some model that aggregates the idea of dependence motivated by repeated measures in time. However, there was homogeneity of variances and covariance at 0, 30, and 60 days, as assessed by Box's  $M$  (Box, 1949; 1950; 1954) test of covariance matrices ( $p = .910$ ). Thus, a Kruskal-Wallis ANOVA test (Huynh and Feldt, 1970) involving 2 treatments x 3 times = 6 groups were conducted and pairwise comparisons were performed using Dunn's procedure (Dunn, 1964) with a Bonferroni correction for multiple comparisons (statistical significance was accepted at the  $p < .0083$  level). Distributions of destructive behavior scores were not similar for all groups, as assessed by visual inspection of box plots. Destructive behavior score was statistically significant different between observed groups ( $\chi^2(5) = 13.262$ ,  $p = .021$ ,  $\eta^2 = .145$ , Cohen's  $d = 0.824$ ,  $\epsilon^2 = .207$ ). Thus, pairwise comparisons were subsequently performed. There were not any significant differences between the placebo and verum-treated group, at any given time (intergroup comparisons) (Figure 1). Only an intra-group difference in verum group was observed from pre- (median = 3.00, mean rank = 43.18) to

post-intervention (median = 2.00, mean rank = 23.07) ( $p = .002$ ; adjusted  $p = .034$ ), one effect of the small sample adopted in this study. The effect of treatment and time on over vocalization and abnormal elimination of feces or urine scores were not statistically significant, and data is shown in Table 5.



**Figure 1** Destructive behavior score. A Kruskal-Wallis ANOVA test involving 2 treatments x 3 times = 6 groups was conducted and pairwise comparisons were performed using Dunn’s procedure with a Bonferroni correction for multiple comparisons ( $p < .0083$  accepted as statistical significance level). Destructive behavior score was statistically significant different between observed groups ( $\chi^2(5) = 13.262, p = .021$ ). Thus, pairwise comparisons were performed subsequently. There were no significant differences between placebo and verum treated group, at any given time (intergroup comparisons). Destructive behavior score is plotted on left y axis: each dot represents an individual score; median is shown as a red line. Mean ranks are plotted on right y axis, and bars denote mean rank for each group. \* denotes the only pairwise comparison that was statistically significant (intragroup comparison).

**Table 5** Behavior analysis of over vocalization and abnormal elimination of urine and feces.

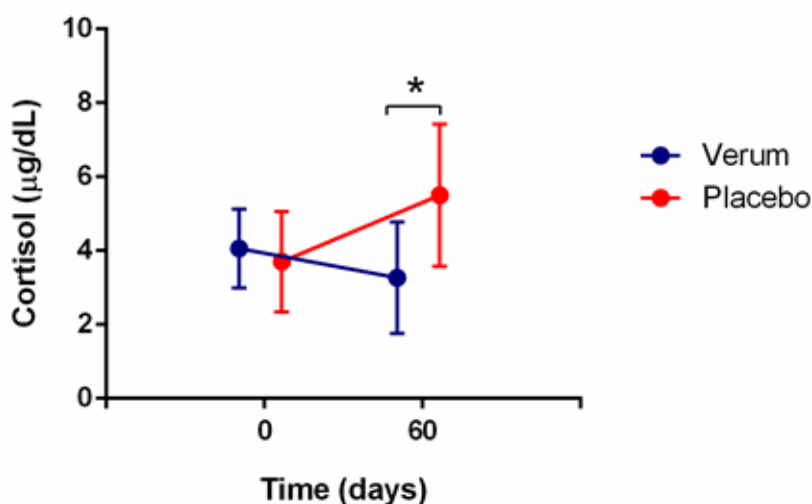
Group	Parameter analyzed	Day	Mean	SE	Mean rank	Kruskal-Wallis stat	p-value
Verum	Over vocalization	0	2.86	.23	37.82	$\chi^2(5) = 7.313$	.198
		30	2.29	.22	26.96		
		60	2.21	.24	26.04		
Placebo	Over vocalization	0	3.14	.34	42.71	$\chi^2(5) = 7.313$	.198
		30	2.57	.20	31.42		
		60	2.57	.37	32.21		
Verum	Abnormal elimination of feces and/or urine in the house	0	2.50	.23	32.07	$\chi^2(5) = 9.198$	.101
		30	2.36	.20	30.35		
		60	1.92	.22	22.00		
Placebo	Abnormal elimination of feces and/or urine in the house	0	2.86	.46	39.14	$\chi^2(5) = 9.198$	.101
		30	2.86	.14	41.07		
		60	2.71	.29	38.93		

There were no statistically significant differences between interventions.



## Plasma analysis

A two-way mixed ANOVA was conducted to investigate the effects of treatment (verum or placebo) and time on cortisol concentrations in the plasma of dogs. There was a statistically significant interaction between treatment and time on cortisol concentration ( $F(1, 19) = 7.858$ ,  $p = .011$ , partial  $\eta^2 = .293$ ), what indicates that the effect of treatment between groups varied in regard to time. Cortisol concentration was significantly greater in the placebo group (mean difference = 2.274,  $SE = 0.998 \mu\text{g/dL}$ ,  $p = .034$ , partial  $\eta^2 = .215$ , Cohen's  $d = 1.055$ ) compared to verum treated dogs after a 60-day treatment program, corresponding to a 72% mean increase, approximately (Figure 2). There was no significant difference between placebo and verum treated groups at day 0 (mean difference =  $-0.366 \mu\text{g/dL}$ ,  $SE = .501 \mu\text{g/dL}$ ,  $p = .473$ , partial  $\eta^2 = .027$ , Cohen's  $d = 0.339$ ). There was no significant interactions, neither at baseline nor to post-intervention, on leukocyte number ( $F(1, 19) = .371$ ,  $p = .550$ ), and on percentages of lymphocyte ( $F(1, 19) = .176$ ,  $p = .680$ ), and segmented cells ( $F(1, 19) = .032$ ,  $p = .859$ ) when comparing blood samples of verum and placebo groups (Table 6).



**Figure 2** Cortisol concentration. There was a statistically significant interaction ( $p = .011$ ) between treatment and time on cortisol concentration, which indicates that the effect of treatment between groups varied among time. Cortisol concentration was significantly greater in the placebo group (mean difference = 2.274,  $SE = 0.998 \mu\text{g/dL}$ ,  $p = .034$ , partial  $\eta^2 = .215$ ) compared to verum treated dogs after a 60-day treatment program, corresponding to a 72% mean increase. Data are shown as mean and 95% confidence interval. \* denotes statistically significantly differences.

**Table 6** Leukocytes, lymphocytes, and segmented cells on blood

Group	Parameter analyzed	Day	Mean	SE	95%CI	
					lower	upper
<i>Verum</i>	Leukocyte (1000/mm <sup>3</sup> )	0	8292.9	669.7	6891.2	9694.5
		60	8342.9	921.3	6414.5	10271.2
Placebo		0	8057.1	947.0	6075.0	10039.3
		60	7442.9	1303.0	4715.8	10170.0
<i>Verum</i>	Lymphocyte (%)	0	27.79	3.13	21.24	34.33
		60	30.14	3.59	22.62	37.67
Placebo		0	24.14	4.42	14.89	33.40
		60	24.14	5.08	13.50	34.78
<i>Verum</i>	Segmented (%)	0	62.71	3.27	55.86	69.57
		60	61.14	3.70	53.39	68.90
Placebo		0	68.86	4.63	59.17	78.55
		60	68.29	5.24	57.32	79.25

There were no statistically significant interactions between treatment and time for any of the variables above. There were no effects of treatment or time alone

## DISCUSSION

All dogs in this study were home alone for a variable number of hours, and their owners went out at least once a day during the working week. A major drawback was the technical impossibility of detecting exactly how long after the owners left the dogs presented SA signs. It is estimated that nearly 30 minutes later, based on neighborhood complaints, they have endorsed over vocalization. Additionally, there was a variation in the number of departures per day, especially considering the weekend, when the owner's absence rate could be even null. Another significant obstacle was to detect dogs that presented the three prominent SA behavioral disturbances together.

Although an objective assessment of the outcome of behavioral research in dogs might be a tough challenge, the double assessment (veterinary and owner) increases the reliability of the study, benefiting the betterment of evidence. Destructive behavior was the most evident SA sign in dogs, followed by over vocalization and abnormal elimination of feces and urine. Although the intergroup comparisons have not unveiled any differences, a significant reduction of destructive behavior was observed in the verum intra-group analysis (pre- to post-intervention). Dogs with anxiety-related disorders might present apprehension accompanied by great excitement, restlessness, and destructive behavior when they realize they are about to be left alone. In addition, a large part of owners from the verum group reported an improvement in the attitude of their dogs during the study, despite the small size and the inequality of the groups, combined with the continuity of external factors (obstacles to cure).

Surprisingly, two owners from the placebo group reported some behavioral improvement, expressed through a peaceful approach and less anxiety. Placebos are therapeutic interventions with non-specific psychophysiological effects and without specific healing powers. The changes in their routines, the daily care ritual, and the presumptive owner's belief in a successful endpoint due to an effective medicine could have influenced their

perception and their dogs' behavior (Sumegi et al., 2014). Even though the placebo effect might occur independently of the kind of medicine, it is more restricted in animals than in human beings, adding value to randomized veterinary clinical research, highlighting some behavioral effects of diluted preparations in this case. It is worth emphasizing that behavioral conditioning was not encouraged in this study.

Dogs have a wide phenotypical variety due to selective genetic pressure caused by taming (Tiira et al., 2016), which promoted the emergence of dependent breeds, known as cooperative dogs (Pongràcz et al., 2020). These tend to overreact when owners are out of sight and present the lowest outcomes in cognitive bias tests, expressed through extreme barking, moving, and inappropriate elimination of excreta. Predicting and managing a frightening situation activates neuroendocrine responses, and might promote anxiety at different intensity levels (Belzung; Philippot, 2007; Wormald et al., 2017). When potential and frequent threats trigger uncontrolled excitatory stimulus due to a mental approach failure (Konok et al., 2011), a generalized behavioral dysfunction like SA can occur (Ohl et al., 2008). For instance, several triggers, like noises (elevator and voices in the hallway, doorbells, or interphone ringing), may prompt odd behaviors when the dog is home alone.

There is evidence that the hypothalamic-pituitary-adrenal (HPA) axis is the primary neuroendocrine pathway related to distress (Zapata et al., 2016; Gruen et al., 2015) comprising hormonal and immune modulation (Dudley et al., 2015). In this study, the intergroup analysis revealed a significant post-intervention increase in cortisol levels from the placebo group compared to verum-treated dogs. The genotypic approach subtleness of homeopathic preparations (Bishayee et al., 2013; Marotti et al., 2014; Saha et al., 2015; Marzotto et al., 2016; Khuda-Bukhsh, 2018) could alter proteins transcription and expression, favoring cortisol levels fluctuation within the threshold, but promoting bio resilience manifested through a behavioral stability. Supposedly, adapted subjects could better cope with their local environment.

Reporting on man-dog relationships and the cortisol level variation in their members, Schöberl et al. (2017) suggested that this hormonal fluctuation reflects a flexible HPA axis adaptation which, in turn, may represent an ability to handle adverse situations, regardless of behavioral dysfunctions. Dogs can roughly reproduce the emotional state of their owners (Hörvath et al., 2008), so both might present a low cortisol variation capacity when the guardian is considered less social and anxious. Moreover, single events like a usual car drive in an attempt to go to the veterinary clinic, or restraint for blood sample collection seem to influence cortisol levels (Hiby et al., 2006), which does not necessarily bear to behavioral disturbances neither pathological conditions (Ryan et al., 2019). Besides, there was also no significant difference in total blood leukocytes, lymphocytes, and segmented cells throughout the study (Table 6). Briefly, there was no straight relation between SA, plasma components, and homeopathic preparations in this research.

Clinical treatment of SA usually comprises multi-drug therapy. Antidepressants (clomipramine, fluoxetine) and anxiolytics (amitriptyline, diazepam, haloperidol) can be supplied for years if well-tolerated by the dog (Sherman; Mills, 2008). Unfortunately, these may become high-risk individuals that might present unpleasant side effects, such as ataxia, sedation, polyphagia, gastritis, loss of motor skills, restlessness, and even aggressiveness and seizures (Ferreira et al., 2012; Gilbert-Gregory et al., 2016).

High-diluted preparations approach to behavioral and physical signs aim at ensuring the balance of individual homeostasis. It is essential to consider individuality in homeopathic randomized clinical trials since the reactions and physiological processes are different. There are no specific preparations for specific clinical conditions. Therefore, supposed suffering would not match with peaceful and calm dogs and their willingness to feed, sleep, and play, according to some owners, even when the dogs are home alone. Hence, the possibility of handling this behavioral disturbance well and without harmful side effects was highly significant to justify this study. Considering the results, more research with a larger sample is required as to highlight the benefits of the treatment of SA with the prescription of individual high-diluted preparations.

## CONCLUSION

The positive outcome in response to destructive behavior refers to a possible bio resilience process generated by the homeopathic medicine, adapting one to better cope with their environment. Despite the auspicious report of some owners, there are many uncertainties regarding the cross-talk between high-diluted medicines, plasma components, and behavioral disturbances in dogs. Studies linking animal welfare, hormones, and feelings in domestic dogs still lack. Nevertheless, experiments with a larger sample could be elucidative.

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## AUTHOR CONTRIBUTIONS

Adalberto von Ancken e Cidéli Coelho were the creators of this project. All three authors participated in the experimental design and writing of the article. Giovanni was responsible for the statistical analysis. The practical part was developed by Adalberto.

## CONFLICT OF INTEREST

Conflict of interest: None. The authors received no corporate financial support for this research.

## REFERENCES

- Appleby, D., Pluijmakers, J., 2003. Separation anxiety in dogs. the function of homeostasis in its development and treatment. *Vet. Clin. N. Am. Small. Anim. Pract.* 33(2), 321-344.
- Belzung, C., Philippot P., 2007. Anxiety from a phylogenetic perspective: is there a qualitative difference between human and animal anxiety?. *Neur. Plast.* 2007, 59676.
- Bishayee, K., Sikdar, S., Khuda-Bukhsh, A. R., 2013. Evidence of an epigenetic modification in cell-cycle arrest caused by the use of ultra-highly diluted *Gonolobus condurango* extract. *J. Pharmacopunct.* 16(4), 7-13.
- Blackwell, E., Casey, R.A., Bradshaw, J.W.S., 2006. Controlled trial of behavioral therapy for separation-related disorders in dogs. *Vet. Rec.* 158(16), 551-554.
- Box, G.E.P., 1949. A general distribution theory for a class of likelihood criteria. *Biometrika.* 36(3-4), 317 – 346.
- Box, G.E.P., 1950. Problems in the analysis of growth and wear curves. *Biometrics.* 6(4), 362-389.
- Box, G.E.P., 1954. Some theorems on quadratic forms applied in study of analysis of variance problems: II – Effects of inequality of variance and correlation between errors in the two-way classification. *Ann. Math. Stat.* 25(3), 484-498.
- Cohen, J., 1988. *Statistical power analysis for the behavioral sciences*, 2nd Edition. Routledge, New York.
- Corty, E.W., 2016. *Using and interpreting statistics: a practical text for the behavioral, social, and health sciences*, 3rd Edition. Worth Publishers, New York.
- Cottam, N., Dodman, N.H., Moon-Fanelli, A.A., Patronek, G.J., 2008. Comparison of remote versus in-person behavioral consultation for treatment of canine separation anxiety. *J. Appl. Anim. Welf. Sci.* 11(1), 28-41.
- 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*). *Vet J.* 177(1), 80-88.
- Dudley, E.S., Schiml, P.A., Hennessy, M.B., 2015. Effects of repeated petting sessions on leukocyte counts, intestinal parasite prevalence, and plasma cortisol concentration of dogs housed in a county animal shelter. *J. Am. Vet. Med. Assoc.* 247(11), 1289-1298.
- Dunn, O.J., 1964. Multiple comparisons using rank sums. *Technometrics.* 6, 241-252.
- Espírito-Santo, H.M.A., Daniel, F., 2018. Calculating and reporting effect sizes on scientific papers (3): guide to report regression models and ANOVA effect sizes. *Port. J. Behav. Soc. Res.* 4(1), 43-60.
- Ferguson, C. J., 2009. An effect size primer: A guide for clinicians and researchers. *Prof. Psychol. Res. Pr.* 40(5), 532–538.
- Ferreira, P.B., Santos, I.M.S., Freitas, R.M., 2012. Aspectos farmacológicos, efeitos anticonvulsivantes e neuroprotetores da buspirona. *J. Appl. Pharm. Sci.* 33(2), 171-179.
- Field, A., 2013. *Discovering statistics using IBM SPSS statistics*, 4th Edition. SAGE, Los Angeles.
- Flannigan, G., Dodman, N.H., 2001. Risk factors and behaviors associated with separation anxiety in dogs. *J. Am. Vet. Med. Assoc.* 219(4), 460-466.
- Gilbert-Gregory, S.E., Stull, J.W., Rice, M.R., Herron, M.E., 2016. Effects of trazodone on behavioral signs of stress in hospitalized dogs. *J. Am. Vet. Med. Assoc.* 249(11), 1281-1291.
- Gruen, M.E., Case, B.C., Foster, M.L., Lazarowskib, L., Fish, R.E., Landsberg, G., DePuy, V., Dorman, D.C., Sherman, B.L., 2015. The use of an open field model to assess sound-induced fear and anxiety associated behaviors in Labrador retrievers. *J. Vet. Behav.* 10(4), 338-345.
- Hahnemann, S., 2007. *Organon of medicine*, 6th Edition. Bento Mure, Brazil. (In Portuguese)
- Hiby, E.F., Rooney, N.J., Bradshaw, J.W.S., 2006. Behavioral and physiological responses of dogs entering re-homing kennels. *Physiol. Behav.* 89(3), 385-391.
- Horváth, Z., Dóka, A., Miklósi, A., 2008. Affiliative and disciplinary behavior of human handlers during play with their dog affects cortisol concentrations in opposite directions. *Horm. Behav.* 54(1), 107-114.



- Huynh, H., Feldt, L., 1970. Conditions under which mean square ratios in repeated measurements designs have exact F-distributions. *J. Am. Stat. Assoc.* 65, 1582-1589.
- Kerepesi, A., Dóka, A., Miklósi, Á., 2015. Dogs and their human companions: the effect of familiarity on dog-human interactions. *Behav. Processes.* 110, 27-36.
- Khuda-Bukhsh, A.R., 2018. Ultra-Highly diluted homeopathic remedy arnica montana 30C can reduce UV-induced DNA damage in *Escherichia coli* through its regulatory influence on nucleotide excision repair genes: a commentary on our published research finding. *Biomed. J. Sci. Tech. Res.* 6(3), 5294-5297.
- Konok, V., Dóka, A., Miklósi, A., 2011. The behavior of the domestic dog (*Canis familiaris*) during separation from and reunion with the owner: a questionnaire and an experimental study. *Appl. Anim. Behav. Sci.* 135(4), 300-308.
- Laerd Statistics, 2017. Two-way ANOVA using SPSS Statistics. Statistical tutorials and software guides. Available online: <https://statistics.laerd.com/>
- Landsberg, G.M., Mougeot, I., Kelly, S., Milgram, N.W., 2015. Assessment of noise-induced fear and anxiety in dogs: Modification by a novel fish hydrolysate supplemented diet. *J. Vet. Behav.* 10(5), 391-398.
- Lees, P., Chambers, D., Pelligand, L., Toutain, P.-L., Whiting, M., Whitehead, M.L., 2017. Comparison of veterinary drugs and veterinary homeopathy: part 1. *Vet. Rec.* 181(7), 170-176.
- Lees, P., Chambers, D., Pelligand, L., Toutain, P.-L., Whiting, M., Whitehead, M.L., 2017. Comparison of veterinary drugs and veterinary homeopathy: part 2. *Vet. Rec.* 181(8), 198-207.
- Marotti, I., Betti, L., Bregola, V., Bosi, S., Trebbi, G., Borghini, G., Nani, D., Dinelli, G., 2014. Transcriptome profiling of wheat seedlings following treatment with ultra-high diluted arsenic trioxide. *Evid. Based. Complementary Altern. Med.* 2014, 851263.
- Marzotto, M., Bonafini, C., Oliosio, D., Baruzzi, A., Bettinetti, L., Di Leva, F., Galbiati, E., Bellavite, P., 2016. Arnica montana stimulates extracellular matrix gene expression in a macrophage cell line differentiated to wound-healing phenotype. *PLOS ONE.* 11(11), e0166340.
- McCrave, E.A., 1991. Diagnostic criteria for separation anxiety in the dog. *Vet. Clin. N. Am. Small Anim. Pract.* 21(2), 247-255.
- McGreevy, P.D., Masters, A.M., 2008. Risk factors for separation-related distress and feed-related aggression in dogs: additional findings from a survey of Australian dog owners. *Appl. Anim. Behav. Sci.* 109(2-4), 320-328.
- McMillan, F.D., Serpell, J.A., Duffy, D.L., Masaoud, E., Dohoo, I.R., 2013. Differences in behavioral characteristics between dogs obtained as puppies from pet stores and those obtained from noncommercial breeders. *J. Am. Vet. Med. Assoc.* 242(10), 1359-1363.
- Nicks, B., Vandenheede, M., 2014. Animal Health and welfare: equivalent or complementary?. *Rev. Sci. Tech.* 33(1), 97-101.
- Ohl, F., Arndt, S.S., Van der Staay, F.J., 2008. Pathological anxiety in animals. *Vet. J.* 175(1), 18-26.
- 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. *J. Am. Vet. Med. Assoc.* 219(4), 467-473.
- 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. *J. Am. Vet. Med. Assoc.* 219(4), 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. *J. Vet. Behav.* 1(3), 124-141.
- Palestrini, C., Minero, M., Cannas, S., Rossi, E., Frank, D., 2010. Video analysis of dogs with separation-related behaviors. *Appl. Anim. Behav. Sci.* 124(1-2), 61-67.
- Parthasarathy, V., Crowell-Davis, S., 2006. Relationship between attachment to owners and separation anxiety in pet dogs (*Canis lupus familiaris*). *J. Vet. Behav.* 1(3), 109-120.
- PNS – National Health Plan, 2013. Brazilian Institute of Geography and Statistics (IBGE). Federal Government, Brazil.
- Pongrácz, P., Gómez, S.A., Lenkei, R., 2020. Separation-related behavior indicates the effect of functional breed selection in dogs (*Canis familiaris*). *Appl. Anim. Behav. Sci.* 222, 104884.



- Ribeiro Filho, A., 2010. Repertório de homeopatia. Editora Organon, Brazil.
- Russell, N.J., Foster, S., Clark, P., Robertson, I.D., Lewis, D.A., Irwina, P.J., 2007. Comparison of radioimmunoassay and chemiluminescent assay methods to estimate canine blood cortisol concentrations. *Aust. Vet. J.* 85(12), 487-494.
- Ryan, M.G., Storey, A.E., Anderson, R.E., Walsh, C.J., 2019. Physiological indicators of attachment in domestic dogs (*Canis familiaris*) and their owners in the strange situation test. *Front. Behav. Neurosci.* 13, 162.
- Saha, S.K., Roy, S., Khuda-Bukhsh, A.R., 2015. Ultra-highly diluted plant extracts of *Hydrastis canadensis* and *Marsdenia condurango* induce epigenetic modifications and alter gene expression profiles in HeLa cells in vitro. *J. Integr. Med.* 13(6), 400-411.
- Sargisson, R.J., 2014. Canine separation anxiety: strategies for treatment and management. *Vet. Med. (Auckl)*. 30(5), 143-151.
- Schöberl, I., Beetz, A., Solomon, J., Wedl, M., Gee, N., Kotrschal, K., 2016. Social factors influencing cortisol modulation in dogs during a strange situation procedure. *J. Vet. Behav. Clin.* 11, 77-85.
- Schöberl, I., Wedl, M., Beetz, A., Kotrschal, K., 2017. Psychobiological factors affecting cortisol variability in human-dog dyads. *PLoS ONE*. 12(2), e0170707.
- Schwartz, S., 2003. Separation anxiety syndrome in dogs and cats. *J. Am. Vet. Med. A.* 222(11), 1526-1532.
- Sherman, B.L., Mills, D.S., 2008. Canine anxiety and phobias: an update on separation anxiety and noise aversions. *Vet. Clin. N. Am. Small Anim. Pract.* 38(5), 1081-1106.
- Sherman, B.L., 2008. Separation anxiety in dogs. *Compend. Contin. Educ. Vet.* 30(1), 27-42.
- Storengen, L.M., Boge, S.C.K., Strøm, S.J., Løberg, G., Lingaas, A., 2014. A descriptive study of 215 dogs diagnosed with separation anxiety. *Appl. Anim. Behav. Sci.* 159, 82-89.
- Sümeği, Z., Gácsi, M., Topál, J., 2014. Conditioned placebo effect in dogs decreases separation-related behaviors. *Appl. Anim. Behav. Sci.* 159, 90-98.
- Takeuchi, Y., Houpt, K.A., Scarlett, J.M., 2000. Evaluation of treatment for separation anxiety in dogs. *J. Am. Vet. Med. Assoc.* 217(3), 342-345.
- Temesi, A., Turcsán, B., Miklósi, A., 2014. Measuring fear in dogs by questionnaires: an exploratory study toward a standardized inventory. *Appl. Anim. Behav. Sci.* 161, 121-130.
- Tiira, K., Lohi, H., 2015. Early life experiences and exercise associate with canine anxieties. *PLoS ONE*. 10(11), e0141907.
- Tiira, K., Sulkama, S., Lohi, H., 2016. Prevalence, comorbidity, and behavioral variation in canine anxiety. *J. Vet. Behav.* 16, 36-44.
- Tomczak, M., & Tomczak, E., 2014. The need to report effect size estimates revisited. An overview of some recommended measures of effect size. *Trends Sport Sci.* 1(21), 19-25.
- White, N., Mills, D., Hall, S., 2017. Attachment style is related to quality of life for assistance dog owners. *Int. J. Environm. Res. Public Health.* 14(6), 658.
- Wormald, D., Lawrence, A.J., Carter, G., Fisher, A.D., 2017. Reduced heart rate variability in pet dogs affected by anxiety-related behavior problems. *Physiol. Behav.* 168, 122-127.
- Zapata, I., Serpell, J.A., Alvarez, C.E., 2016. Genetic mapping of canine fear and aggression. *BMC genomics.* 17, 572.

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