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Training effects on dog behavior

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Dog trainers free lancers

Abstract: The purpose of this paper has been to investigate whether there are behavioral differences, and therefore repercussions on dog welfare, related to having attended or not training course and the characteristics of the latter. A questionnaire was proposed to a sample of dog owners regardless of their previous experience in dog training. The first section of the questionnaire regarded the personal data of the owner and the dog, the second section examine the daily dog management and its habits, a third section concerned the dog training and in a final part, 44 multiple-choice questions about dog behavior and their frequency of display (often, sometimes or never) were asked.

A total of 153 questionnaires were collected, half represent trained dogs and the other half represent untrained dogs. The statistical analysis revealed that certain behavioral problems are more observable in untrained dogs, like urinating at home ($\chi^2 = 6.445$; $p = 0.011$), excessive excitability when the owners return ($\chi^2 = 5.112$; $p = 0.024$), jumping on the owners in different circumstances than when they return home ($\chi^2 = 6.115$; $p = 0.013$), pulling the leash ($\chi^2 = 4.567$; $p = 0.033$). It was also shown that the duration of the training and the number of sessions in which it took place also influenced the manifestation of these behaviors. Finally, differences in behavior were observed due to the method used within the subgroup of trained dogs. This preliminary investigation show that the training dogs have less behavioral problem than the untrained dogs.

Key Words: dog training; dog welfare; behavioral problems.

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Introduction

The advancement of urbanisation and the increase in the number of pets in urban areas have aroused the interest of scientists in exploring the social interactions between humans and animals and the effects that these interactions have on animal welfare (Ruis et al., 2001; Mariti et al., 2015). Over time, the primary function of dogs has changed from utility animals to pets and the social interaction between dogs and humans has become a topic of great interest both in the popular literature (Serpell, 1995) and, more recently, in the scientific literature (Miklósi et al., 2004; Steinker, 2007; Bradshaw et al., 2009; Gazzano et al., 2013).

Most owners live with their pets for a long time, allowing the formation of a stable long-term relationship (Mariti et al., 2013a,b; 2014). The relationship between dog and owner is a vital aspect of the dog's welfare and this bond also influences the owner's well-being (Crawford et al., 2006).

The term "behavioral problem" is used to describe any behavior shown by a pet that is unacceptable to its owner (Amat et al., 2009; Mengoli et al., 2013).

Behavioral problems can be normal behaviors such as barking or abnormal behaviors such as stereotypies. Both types of behavior can become problematic for the dog owner and for society. One consequence can be a reduction of welfare for the dog showing these behaviors (Stafford, 2008). Indeed, behavioral problems can indirectly influence a dog's welfare through the owners'

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reaction and response to the behaviors shown (Stafford, 2008), demonstrating the deep impact that owners have on the welfare of their dogs. Canine behavioral problems can break the bond between dog and owner, sometimes leading to dog abandonment in kennels (Patronek et al., 1996) and, in extreme cases, to dog euthanasia (Patronek et al., 1995; Houpt et al., 1996).

It has been shown that dog owners who spend time and perform activities with their dog, such as training and walking, observe fewer behavioral problems in their dogs (Bennett & Rohlf, 2007; Zilocchi et al., 2016). However, in modern society many owners work full time leaving their dog home alone for most of the day, which can lead to anxiety and behavioral problems related to separation (Rugbjerg et al., 2003; Rehn & Keeling, 2011; Scaglia et al., 2013).

The domestic dog is a highly social species capable of complex communication with conspecifics (Mariti et al. 2017; Gazzano et al., 2014), and people (Hare & Tomasello, 2005), for instance by associating subtle visual signals of their owners with positive or negative outcomes (Rooney et al., 2001; Cullinan et al., 2004). Therefore, it is not surprising that differences in owners' approaches to manipulation and training seem to influence the onset of unwanted behavior in their dogs (Hiby et al., 2004).

Dog training is essential for the domestic dog to be prepared to live better and with fewer problems of adaptation in the home and in the society of humans (Fanoni, 2003).

Animal welfare is a relevant topic for dog training in several ways, for example coercive techniques can cause fear and stress (Ziv, 2017). Numerous studies show the negative effects of the use of coercive training techniques such as aggression, stress and behavioral problems (Arhant et al., 2010, Blackwell et al., 2008, Casey et al., 2014, Cooper et al., 2014, Herron et al., 2009, Hiby et al., 2004; Masson et al., 2018) which may have implications for the human-animal bond and for future training as well as compromise the mental and physical health of dogs (Ziv, 2017).

On the contrary, it has been shown that well-conducted training increases obedience and decreases behavioral problems in dogs (Bennett & Rohlf, 2007; Clark & Boyer, 1993; Jagoe & Serpell, 1996). This means that training based on positive reinforcement can contribute to welfare as an enrichment activity for dogs and in reinforcing the bond between humans and dogs by allowing them to understand each other and communicate correctly.

Therefore, the aim of this study is to investigate if dog training can affect dog behavior improving obedience and allowing the bonding of dog-owner relationship. For this purpose, behavioral differences between trained and untrained dogs have been analysed to determine the effects of dog training.

Materials and methods

For the following research a questionnaire, divided into 4 sections, was used. The questionnaire was distributed online on Facebook in order to ensure a heterogeneous sample.

The first section of the questionnaire regarded the personal data of the owner (age, sex, level of education and profession) and the dog (sex, age, reproductive status, origin), the second section examine the daily dog management (possibility to go out in the garden, presence of other animals in the house, etc..) and its habits (play, walks, etc.), a third section concerned the dog training (duration and number of sessions, method used, professional qualification of the trainer, etc.) and in a final part, 44 multiple-choice questions about dog behavior and their frequency of display (often, sometimes or never) were asked.

After collecting data, the statistical analysis was obtained through the chi-squared test which uses the chi-squared distribution (χ^2). P-values lower than 0.05 were considered statistically significant.

Results and discussion

General data:

Among the 153 dog owners who participated in the questionnaire, 80 (52%) attended a dog training course while the remaining 73 (48%) never attended a dog training course. The average age of the owners who filled in the questionnaire is 33.5 years ($\sigma = \pm 14$) and the prevailing sex is female (83.7%).

Most of the dogs that compose the sample examined are cross breed, while all the pure breeds are poorly represented. The average age of the sample was 6.1 years ($\sigma = \pm 37.35$) with a minimum of 2 years and a maximum of 17 years. Indeed, all questionnaires reporting ages under 24 months were rejected to exclude non-adult subjects from the sample. Regarding sex distribution, even though males and females are equally distributed, 77.6% of females are sterilized while only 28.6% of males are.

39.2% of the owners stated they had never owned a dog before the current one. This data could have affected the results of this survey as separation and other behavioral problems have often been associated with the owners being at their first experience (Jagoe & Serpell, 1996; Ledger, 2000). Owners who have no experience of managing and communicating with dogs may in fact respond inappropriately to canine behavior patterns and may inadvertently initiate, or enhance, problematic behavior (Peachey, 1993).

Behavioral data:

Training - Regarding the differences between trained dogs and untrained dogs (Fig. 1), it has been observed that untrained dogs show more often the following behaviors: urinating at home ($\chi^2 = 6.445$; $p = 0.011$), excessive excitability when the owners return ($\chi^2 = 5.112$; $p = 0.024$), jumping on the owners in different circumstances than when they return home ($\chi^2 = 6.115$; $p = 0.013$), pulling the leash ($\chi^2 = 4.567$; $p = 0.033$). These behaviors are ascribable to an incorrect approach of the owner and therefore solvable and above all preventable through training. In fact, dogs mainly jump on people who are familiar with them and this behavior is influenced by the position and posture of the person (Rezac et al., 2017). Moreover, even if this is an undesirable behavior, people recognize jumping as friendly behavior and often reinforce it involuntarily (Pirner & McGlone, 2016). As for house soiling, it is possible that the owners do not spend enough time on walks with their dogs. It is therefore useful to attend training courses to understand how to prevent these behavioral problems and to reinforce the correct behavior (Gazzano et al., 2008), as demonstrated also in other species (Gazzano et al., 2015). The lack of adequate training and spending too much time alone are in fact factors that influence house soiling (Chung et al., 2016). In addition, dogs that have been forced to soil in confined spaces for a long time may lose their natural tendency to eliminate outside the home, or dogs that have always lived outdoors often do not learn to distinguish between indoor and outdoor environments (Overall, 2013). As far as the conduct on a leash is concerned, it is also manageable through a correct training and habituation. Moreover, very often pulling on the leash behavior has been studied to be associated with other behaviors, all of which are symptoms of excessive excitability, such as the one previously mentioned of jumping on people (Bennett & Rohlf, 2007; Shabelansky & Dowling-Guyer, 2016).

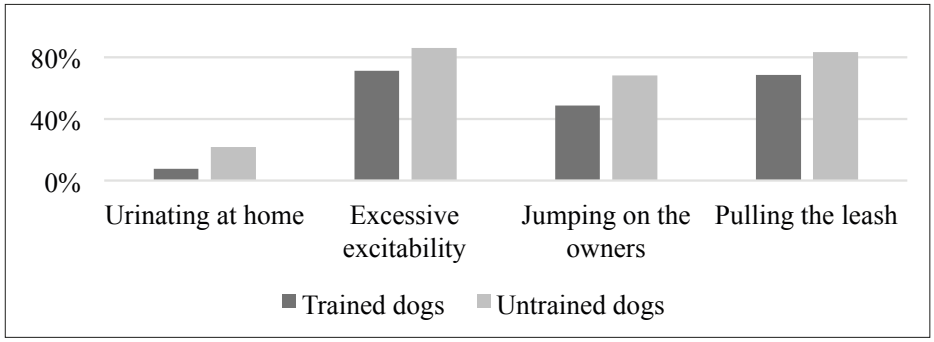

Fig. 1. Behavioral differences between trained and untrained dogs

Training duration - As for the differences related to the duration and number of sessions of the training course, it was found that some behaviors are more frequent among dogs who have attended shorter courses or courses consisting of fewer sessions. In particular, among dogs who have attended a training course lasting less than 6 months, it is more common to observe behaviors such as urine house soiling ($\chi^2 = 4.199$; $p = 0.040$), coprophagy ($\chi^2 = 4.409$; $p = 0.036$), aggressive attitudes when touched on the head ($\chi^2 = 3.827$; $p = 0.050$) and not like the entry of people into their territory ($\chi^2 = 0.678$; $p = 0.017$). In dogs who attended a training course in less than 5 sessions, on the other hand, behaviors such as faeces house soiling ($\chi^2 = 7.002$; $p = 0.008$) and fear of the vet ($\chi^2 = 4.347$; $p = 0.036$) were found. There are also common behaviors both for dogs who have attended shorter courses and for dogs who have attended fewer training sessions. In particular, the following behaviors were significantly more common: turning on itself ($\chi^2 = 4.762$; $p = 0.029$ for dogs who attended courses of less than 6 months and $\chi^2 = 4.945$; $p = 0.026$ for those who attended less than 5 sessions), repeating some action insistently ($\chi^2 = 6.574$ and $p = 0.010$ for dogs who attended courses of less than 6 months and $\chi^2 = 9{,}924$ and $p = 0.002$ for those who have attended less than 5 sessions) and aggressive behavior when scolded ($\chi^2 = 5.678$ and $p = 0.017$ for dogs who have attended courses lasting less than 6 months and $\chi^2 = 3.634$ and $p = 0.057$ for those who have attended less than 5 sessions). From the observation of these results we could deduce that attending training courses for a longer time could improve communication with dogs and consequently reduce their behavioral problems.

Training method - In the present research, statistically significant differences in behavior emerge between dog trained with gentle method and dog trained with coercive method (Fig.2). In particular, it was more common among coercively trained dogs to show the following behaviors: defecating in the house ($\chi^2 = 5.098$; $p = 0.024$), self-licking insistently ($\chi^2 = 3.964$; $p = 0.046$) and defending one or more objects (e. g. toys, food bowl) ($\chi^2 = 3.909$; $p = 0.048$). In our samples only a small percentage of the sample (10%) used coercive methods and these data are therefore not representative of the population. However, these results are in line with other researches. Indeed, according to a study conducted in 2008 by Blackwell et al., there is a significant relationship between the training methods used by owners and the inappropriate behavior shown by dogs. The training methods used are also significantly associated with the level of seeking attention, fear and aggressiveness (Blackwell et al., 2008). It has also been studied that dogs trained with negative reinforcements show more stress-related behavior than dogs trained with positive reinforcements (Deldalle & Gaunet, 2014). Several studies show that the immediate effects of the coercive approach may include behavioral signs related to fear and stress (Beerda et al., 1998; Schalke et al., 2007; Schilder & van der Borg, 2004) but also aggressive reactions (Herron et al., 2009). Persistent licking is a behavior often used as a substitute activity and has been studied to be a symptom of stress (Beerda et al., 1999; Hetts et al., 1992). Moreover, since pet dogs do

not have to compete for survival and reproduction, they do not need to defend resources from a functional point of view (Bradshaw et al., 2016). However, resource defence behavior is still observed (Guy et al., 2001) in varying grades and types and may therefore indicate a stress condition. Finally, house soiling can be a sign of separation anxiety if it occurs in the absence of the owner (Flannigan & Dodman, 2001).

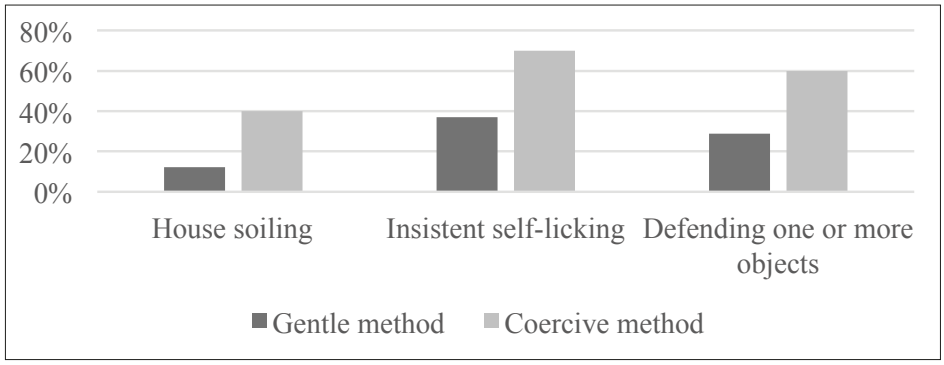

Fig. 2. Behavioral differences between dogs trained with gentle or coercive method

Punishments and rewards – In our study, the use of punishments was not found to be related to the occurrence of undesirable behavior. On the contrary, it has been observed that dogs educated by punishments, such as shouts and blows, present less frequently behaviors of eating anything on the street ($\chi^2 = 5.305$; $p = 0.021$) and chasing cats ($\chi^2 = 4.478$; $p = 0.034$). This result may be due to the inhibition that punishments can create when they are imposed on dogs. Indeed, during risk assessment, non-defensive behavior, self-grooming, nutrition and social interaction are inhibited (Blanchard et al., 1998; Mastripieri et al., 1992; Shuhama et al., 2007). The extent of inhibition of these behaviors can be used as an indirect index of alertness or anxiety (Shuhama et al., 2007). Exploration can in fact be partially or completely inhibited by anxiety, also in puppies (Guardini et al., 2016; 2017): a reduced exploration can become a measure of anxiety status (Crawley & Goodwin, 1980; Ohl et al., 2008). Instead, the use of rewards in food or game has turned out, as expected, not to be associated with any behavioral problem. On the contrary, the behavior of house soiling ($\chi^2 = 5.773$; $p = 0.016$) among those who declared not to use rewards compared to those who use them was more present. This could be due to the fact that not using the rewards to reinforce the behavior of eliminating outdoor may affect learning.

Conclusions

In conclusion, the findings of this research agree with what is generally reported by other studies. Trained dogs were found to have fewer behavioral problems than untrained dogs. It was also evident that the duration of the training courses affects the results obtained by showing that the courses carried out for longer time and in more sessions guarantee better results. Moreover, although within the sample only a small part of subjects was trained by the coercive method, it was found that the use of this method causes more behavioral problems than the gentle method. Finally, the use of punishments was found to be related to the inhibition of several behaviors while the use of rewards is related to the reduction of house soiling.

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Effetto della partecipazione a corsi di educazione cinofila sul comportamento del cane

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Istruttori cinofili liberi professionisti

Sintesi

Lo scopo di questo lavoro è stato quello di investigare se esistano differenze comportamentali e perciò ripercussioni sul benessere del cane, correlate all'aver seguito o meno un corso di educazione cinofila.

Ad un gruppo di proprietari di cani, senza tener conto delle loro pregresse esperienze, è stato proposto un questionario, suddiviso in 4 sezioni: la prima raccoglieva i dati anagrafici del proprietario, la seconda il management quotidiano del cane, la terza il tipo di corso di educazione cinofila che il cane aveva frequentato ed infine un'ultima parte riguardante il comportamento del cane.

Sono stati raccolti 153 questionari, metà dei quali compilati da proprietari di cani che non avevano seguito corsi di educazione cinofila. L'analisi statistica ha rivelato che alcuni problemi comportamentali sono maggiormente presentati da cani che non hanno frequentato corsi di educazione, come urinare in casa ($\chi^2 = 6{,}445$; $p = 0{,}011$), eccessiva eccitabilità quando i proprietari ritornano a casa ($\chi^2 = 5{,}112$; $p = 0{,}024$), saltare sopra il proprietario in situazioni diverse dal ritorno a casa ($\chi^2 = 6{,}115$; $p = 0{,}013$), tirare al guinzaglio ($\chi^2 = 4{,}567$; $p = 0{,}033$).

La durata del corso di educazione cinofila ed il numero delle sue sessioni, influenzano la manifestazione di questi comportamenti. Questi dati preliminari mostrano che i cani che hanno seguito corsi di educazione cinofila presentano in misura minore problemi comportamentali.

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Blood serotonin concentrations in phobic dogs fed a dissociated carbohydrate-based diet: a pilot study

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Abstract: Aim of this study was to evaluate the effects of a carbohydrate-based diet on serotonin blood concentrations in phobic dogs. For this study were recruited, from a public shelter, three dogs (2 neutered females and 1 male), weighing between 15 and 30 kg and living in the shelter for more than six months. Dogs received by a veterinary behaviorist a diagnosis of interspecific social phobia. The dogs were fed 2 daily meals (at 8.00 A.M. and 4.00 P.M.), the first meal was exclusively carbohydrate-based (puffed rice) whereas the second one was composed by the commercial diet.

Blood was collected every 21 days after 8 hours from carbohydrate meal to determine the levels of serotonin (5-HT), L-tryptophan (TRP) and cortisol. Statistical analysis did not reveal any significative difference between the serum concentrations of 5-HT, TRP and cortisol, at the different times, despite a tendency to increase during the time.

The results of this research are useful for directing further studies in the right direction, verifying the correctness of the hypotheses that can be formulated based on the analysis of these data.

Blood concentrations of cortisol suggest that there have been no particular episodes of stress. For this reason, it is possible to exclude that the reduced transformation of TRP in 5-HT is due to an increased activity of tryptophan 2,3-dioxygenase induced by cortisol. In conclusion, these results are to be considered as a further step to address, more correctly, further research on the effect of diet manipulation on serotonin blood and brain concentrations.

Key Words: serotonin, dog, phobia, dissociate diet, carbohydrates.

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Introduction

Domestic animals, especially the dogs, have shared their lives with humans for a long time (Savolainen et al., 2002). However, this relationship is sometimes negatively influenced by the exhibition of particular aspects of their ethology (Mengoli et al., 2013) or by behavioral problems. In dogs, intra and interspecific aggression, phobias and separation problems are the most frequent behavioral problems and reasons of relinquishment (Patronek et al., 1996).

Between these problems, attention has been recently paid to phobia, for its relevance in to affect dog welfare.

Phobia can be defined as a persistent and excessive fear of an object, individual or situation (Sherman & Mills, 2008). In dogs, different types of this behavioral pathology are signaled: noise, thunderstorm, social phobia and different therapeutic interventions have been proposed.

Phobia and other behavioral problems can result from many causes, including an altered functionality of serotoninergic pathways in the brain, characterized by serotonin (5-HT) deficiency (Sachs et al., 2015; Rosado et al., 2010 a,b). 5-HT and other neurotransmitters, mediating communication between cells and acting as trophic factors in the processes of neurogenesis, derive from precursors introduced with the diet. For this reason, in the last years some researchers have

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increasingly focused their attention on the possible effects of the diet on the behavior (e.g. Gatta et al., 2013).

As regarding the synthesis and release of 5-HT by brain, neurons are rapidly influenced by the local tryptophan (TRP) concentration (Fernstrom et al., 1990; Sharp et al., 1992). TRP passes through the blood–brain barrier by using carriers for which it competes with other large neutral amino acids (LNAAs), mainly leucine, isoleucine, valine, tyrosine and phenylalanine (Oldendorf & Szabo, 1976).

In the brain, TRP is hydroxylated to 5-hydroxytryptophan by the enzyme TRP hydroxylase, that is usually about half saturated with the amino acid (Carlsson, 1978). Therefore, increases in TRP in the brain can maximally double the 5-HT synthesis (Carlsson, 1978), but meals containing protein fail to raise brain TRP concentrations because of the contemporary increase of both serum TRP and LNAA concentrations by proportionally similar amounts, resulting in no net change in competition for uptake (Fernstrom, 1980).

Instead, previous studies have shown that the ingestion of a large carbohydrate, protein-free meal by fasting rats, rapidly raises brain TRP concentrations and stimulates 5-HT synthesis (Fernstrom, 1980). The carbohydrate meal is known to produce its effects on TRP via insulin secretion, which depresses the plasma concentrations of LNAAs in mammals, increasing their uptake into muscle, except TRP due to its bond with albumin (Fernstrom & Wurtman, 1972; Lotspeich, 1949).

Studies performed on dogs have produced contrasting results: De Napoli (De Napoli et al., 2002) found that the addition of TRP to high protein diets or the switch to a low protein diet might reduce aggression in dogs displaying so-called dominance and territorial aggression. Plasma concentrations of 5-HT and TRP had consistent results in all phases of the study, despite different concentrations of dietary TRP. As suggested by the authors, this is most likely due to their inadequate analytic methods. Mugford reported that there was a reduction in aggressive behaviors in three of the seven aggressive Golden Retrievers after the introduction of a low-protein diet (15–18% of total energy) (Mugford, 1987). However, the composition of the experimental and previous diets was not reported.

In another study (Dodman et al., 1996), twelve dogs that exhibited either high territorial aggression dominance aggression or hyperactivity, and fourteen control dogs were fed each of three diets varying in protein content (180, 250 and 310 g crude protein/kg DM) for two weeks at living in-home situations. The low-protein diet and medium-protein diet decreased territorial aggression scores in comparison to the high-protein diet.

Our recent study has demonstrated that, in dog, a carbohydrate-based diet led to a decrease in LNAAs levels and consequently led to a significant higher TRP/LNAAs ratios 6 h after the provision of carbohydrates (Gazzano et al., 2018).

No one, until now, has investigated the effects of carbohydrate-based diets on serum serotonin concentrations, despite a study showed correlations between low levels of serotonin in blood and a behavioral pathology like aggressive behavior in English Cocker Spaniels (Amat et al., 2013).

In addition, serotonin synthetized in the brain can overcome the blood-brain barrier. As demonstrated by Nakatani et al. (2008), 5-HT transporters located on the brain endothelial cells may act as the efflux transport system for the 5-HT that crosses from the brain into the circulating blood.

Aim of this study was to evaluate the effects of a carbohydrate-based diet on serotonin blood concentrations in phobic dogs.

Animals, materials and methods

For this study, approved by the Ethical Committee of the University of Pisa, Italy (protocol n° 38/2016) in accordance with Directive 2010/63/EU, were recruited, from a public shelter, three

dogs (2 neutered females and 1 male), weighing between 15 and 30 kg and living in the shelter for more than six months. Dogs received by a veterinary behaviorist a diagnosis of interspecific social phobia. The study was conducted between January and April 2019.

The animals were fed two meals (at 8.00 A.M. and 4.00 P.M.) for 15 days with a commercial diet to standardize their metabolic status. After this period the dogs were fed 2 daily meals (at 8.00 A.M. and 4.00 P.M.), the morning meal was exclusively a carbohydrate-based one (puffed rice) whereas the evening meal was composed by the commercial diet previously eaten during the adaptation period. Ingredients and diet analytical constituents are reported in Table 1.

Table 1. Ingredients and diet analytical constituents (as fed basis).

		Commercial kibble	Puffed Rice	Diet
Moisture	%	10.0	5.5	7.9
CP	%	28.0	7.5	18.8
Fat	%	21.0	1.0	13.4
CF	%	2.6	2.5	2.5
NSC	%	31.9	83.0	53.0
Ash	%	6.5	0.5	3.8
EM	kcal/kg	3800	3250	3690

Blood was collected every 21 days after 8 hours from carbohydrate meal to determine the levels of serotonin (5-HT), L-tryptophan (TRP) and cortisol. Blood samples (4 ml) were left to coagulate at room temperature for 60 minutes, then centrifuged in ALC 4237R Refrigerated Centrifuge at 7000 rpm for 20' to 4°C to obtain the serum. The serum was divided into 200 µl aliquots and frozen until the time of analysis.

The extraction and quantification of 5-HT and TRP in serum samples were performed following an HPLC method, as previously described in the literature (Bearcroft et al., 1995; Atkinson et al., 2006) and based on fluorimetric detection. This method was slightly modified as follows: 200 µl $HCLO_4$ 4% v/v containing 2mM EDTA were added to 200 µl of serum or standard solution to precipitate proteins; the extract was mixed and centrifuged at 13000 rpm in micro centrifuge (ALC microCENTRIFUGETTE ® 4214) for 3 minutes. 50 µL of supernatant were taken with MICROLITER™ Syringes #705 and 20 µl injected into HPLC for analysis.

HPLC analyses were performed using a RP Gemini C18 column (250 mm x 4.6 mm, 5 um) (Phenomenex, Torrance, CA, USA) and a Jasco HPLC apparatus (Jasco Corporation, Ishikawa-Machi Hachioji-Shi, Tokyo, Japan) equipped with 2 gradient pumps (PU-1580), a mixer unit (HG-2080-03) and a fluorescence detector (FP-920).

The mobile phase consisted of methanol (CH_3OH) and ammonium acetate (CH_3COONH_4) 100 mM (20:250 v/v), pH 4.5, degassed and filtered with 0.2 µm diameter filters and eluted at a flow rate of 0.800 ml/min.

Fluorescence detector was set at 290 nm excitation wavelength and 337 nm emission wavelength. Data was acquired using Jasco Borwin 1.5.0 software (Jasco Corporation, Ishikawa-machi Hachioji-shi, Tokyo, Japan). The interface between chromatography instruments and a PC based data acquisition is the JMBS electronic interface box HERCULE 2000 VI.0.

Serotonin creatinine sulfate monohydrate and L-tryptophan (TRP) were purchased from Sigma-Aldrich Inc. (Saint Louis, MO, USA).

Stock solution (10 mM) of 5-HT and stock solution (100 mM) of TRP were prepared in 10 ml $HClO_4$ 10%, divided in aliquots of 1 ml and stored at -20°C. Diluted standard solutions in

$HClO_4$ 4% were prepared daily and employed to identify chromatographic peaks and to calculate calibration curves.

Cortisol concentrations from canine serum were measured using an ELISA kit (Diametra®, Segrate, Italy), according to the manufacturer's instructions. Briefly, the antigen cortisol in the sample competes with the antigenic cortisol conjugated with horseradish peroxidase-cortisol (HRP) for binding to the limited number of antibodies anti cortisol coated on the microplate. After incubation and washing, the enzyme HRP in the bound fraction reacts with the substrate (H_2O_2) and the TMB substrate and develops a blue color that changes into yellow when the stop solution (H_2SO_4) is added. Cortisol concentration in the sample is calculated based on a series of standards and the color intensity is inversely proportional to the cortisol concentration in the sample. The method allows the determination of cortisol from 0 to 500 ng/ml.

Data were statistically analyzed applying Wilcoxon test, by using SPSS® STATISTICS 17.0.

Results

In figure 1, 2, 3 are reported the serum concentrations of 5-HT, TRP and cortisol of the three dogs examined. Statistical analysis (Table 2) did not reveal any significative difference between the serum concentrations of 5-HT, TRP and cortisol, at the different times, despite a tendency to increase during the time.

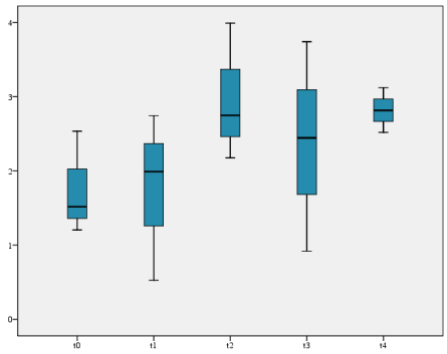

Figure 1. Mean serum 5-HT concentration (μM/ml ± S.D.) at different time (T0=baseline, T1=3 week, T2=6 week, T3=9 week, T4=12 week).

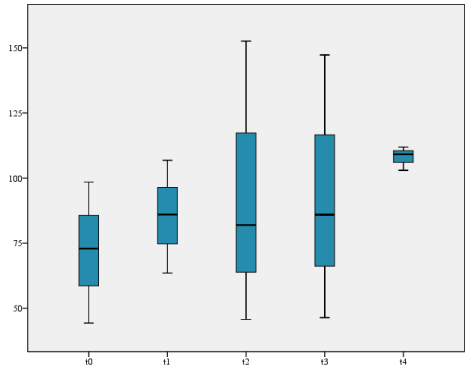

Figure 2. Mean serum TRP concentration (μM/ ml ± S.D.) at different time (T0=baseline, T1=3 week, T2=6 week, T3=9 week, T4=12 week).

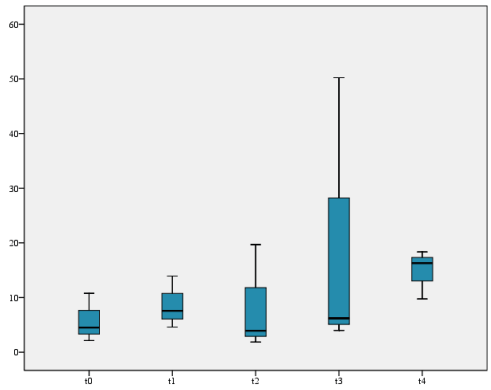

Figure 3. Mean serum cortisol concentration (ng/ml ± S.D.) at different time (T0=baseline, T1=3 week, T2=6 week, T3=9 week, T4=12 week).

Table 2. Z values obtained with Wilcoxon test for 5-HT, TRP and Cortisol, comparing their concentration between different times analyzed.

Z	T1 *vs* T0	T2 *vs* T0	T3 *vs* T0	T4 *vs* T0
5-HT	0; *n.s.*	-1.604; *n.s.*	0; *n.s.*	-1.604; *n.s.*
TRP	-1.604; *n.s.*	-1.604; *n.s.*	-1.604; *n.s.*	-1.604; *n.s.*
Cortisol	-0.535; *n.s.*	0; *n.s.*	0; *n.s.*	-1.604; *n.s.*

Discussion

Phobia is one of the most serious behavioral problem in dogs because this pathology can deeply affect the quality of life, limiting the possibility of this animal to participate to the activities of the owner.

Among the different forms of phobia, the social, interspecific, towards people, is certainly the most serious for its consequences on the relationship with the human being and for the difficulty of management of the animal that it entails. If a dog that presents a phobia of noises can, with special care, be managed in an acceptable way for its welfare, this becomes practically impossible in the case of interspecific social phobia. In fact, the dog lives in a strongly anthropized social environment in which it is necessary that it can fit properly.

The causes of social phobia are certainly due to deficiencies during the development of the behavior of the puppy that has not been adequately socialized towards the human being. Since Scott & Fuller's pioneering researches in the sixty (Scott & Fuller, 1965), we know that in the period of socialization, puppies must be exposed to the greatest possible number of stimuli (Gazzano et al., 2008a).

Maternal care (Guardini et al., 2015; 2016; 2017) and neonatal stimulations (Gazzano et al., 2008b) can also have a positive effect in a normal development of the puppies.

However, it has been pointed out that phobia could have genetic bases. Some studies have, in fact, found a greater incidence of phobia, especially regarding noise, in certain breeds (Mengoli et al., 2012). Moreover, the standards of some breeds, that carry generic annotations regarding suspiciousness towards strangers, make to suppose that diffidence, if not a true phobia, is typical and, in the past, sought after as it is considered positive for the working use of the dog.

The manipulation of the diet can be an easy tool to use by the owner and well accepted even by people who exclude the use of psychotropic drugs in the therapy of behavioral pathologies of their animal.

It is therefore of considerable interest to check whether there is the possibility of using special diets to change the behavior of the dog.

The results of previous research have shown a link between serotonin blood levels and behavioral pathologies, such as aggression (Amat et al., 2013), a behavior often present in interspecific social phobia. Other studies conducted also by our research group (Gazzano et al., 2018), have shown how the manipulation of the diet can modify the blood relationship between TRP and other neutral amino acids, thus increasing its bioavailability.

The data of this first preliminary research does not clarify whether the increased bioavailability of TRP contributes to increasing the synthesis of serotonin. In fact, serotonin concentrations, although showing a tendency to increase with respect to the initial value, did not present statistically significant variations between the different times considered.

The major limitation of this study obviously lies in the reduced number of subjects examined, even though they constitute a homogeneous group with regards to living environment, time spent in shelters, management and diet.

However, these results are useful for directing further studies in the right direction, verifying the correctness of the hypotheses that can be formulated based on the analysis of these data.

Blood concentrations of cortisol, which are stable during the period examined, suggest that there have been no episodes of stress. This result is of particular importance because it allows us to hypothesize that the reduced transformation of TRP in 5HT is not due to an increased activity, in the liver, of tryptophan 2,3-dioxygenase induced by cortisol. In fact, in mammals more than 90% of the total TRP is degraded in the liver through the kynureninase pathway (Young et al., 1978) and the first enzyme of tryptophan oxidation, tryptophan 2,3-dioxygenase, is regulated by glucocorticoids (Knox & Mehler, 1951; Voigt & Sekeris, 1980) and by tryptophan itself (Knox, 1951, 1966). In an in-vitro experiment with rat liver cells, replacement of glucocorticoid with low doses of dexamethasone phosphate resulted in a 7-8-fold increase in the total activity of tryptophan 2,3-dioxygenase (Salter & Pogson, 1985).

Further studies will be necessary to rule out, with certainty, this hypothesis and to verify, instead, if the lack of increase in the production of serotonin depends on an insufficient passage of TRP through the blood-brain barrier, caused by a reduced presence of the amino acid in the carbohydrate-based diet: the addition of TRP to the diet will allow us to evaluate the validity of this hypothesis.

Finally, it will be necessary to verify if the little increase in blood serotonin, observed at the different times of collection and maintained for three weeks, even after the suspension of the diet, is due to the positive effect of the exposure of the animals to a photoperiod longer. Bright light is, in fact, a standard treatment for human seasonal depression, but some studies also suggest that it is an effective treatment for nonseasonal depression (Golden et al., 2005) and also reduces depressed mood in women with premenstrual dysphoric disorder (Lam et al. 1999), and in pregnant women suffering from depression (Epperson, 2004).

In human postmortem brain, serotonin levels are higher in those who died in summer than in those who died in winter (Carlsson, 1980). A similar conclusion came from a study on healthy volunteers, in which serotonin synthesis was assessed by measurements of the serotonin metabolite 5-hydroxyindoleacetic acid in the venous outflow from the brain (Lambert et al., 2002). There was also a positive correlation between serotonin synthesis and the hours of sunlight on the day the measurements were made, independent of season.

In conclusion, these results are to be considered as a first step to address, more correctly, further research on the effect of diet manipulation on serotonin blood and brain concentrations.

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Concentrazioni ematiche di serotonina in cani fobici alimentati con una dieta dissociata a base di carboidrati: uno studio pilota

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Sintesi

Lo scopo di questo studio è stato quello di valutare gli effetti di una dieta a base di carboidrati sulla concentrazione ematica in cani fobici. Per questo studio sono stati reclutati da un canile pubblico, 3 cani (2 femmine castrate ed 1 maschio), di peso compreso tra 15 e 30 Kg e ospitati in canile da almeno 6 mesi. Ai cani è stata diagnosticata, da un veterinario esperto in comportamento, una fobia sociale interspecifica. I cani sono stati alimentati con 2 pasti giornalieri (alle 8,00 e alle 16,00), il primo esclusivamente a base di carboidrati (riso soffiato) ed il secondo composto dalla dieta commerciale con cui erano solitamente alimentati.

Il sangue è stato prelevato ogni 21 giorni, dopo 8 ore dal pasto di carboidrati per determinare i livelli di serotonina, L-triptofano e cortisolo.

L'analisi statistica non ha rivelato alcuna differenza statisticamente significativa tra le concentrazioni di serotonina, L-triptofano e cortisolo, ai differenti tempi analizzati, nonostante una tendenza all'aumento durante il tempo.

I risultati di questa ricerca sono utili per indirizzare ulteriori studi nella giusta direzione, verificando la correttezza delle ipotesi che possono essere formulate sulla base dell'analisi di questi dati.

Le concentrazioni ematiche di cortisolo suggeriscono che non ci siano stati particolari episodi di stress. Per questa ragione è possibile escludere che la ridotta di triptofano in serotonina sia dovuta ad un'aumentata attività della triptofano-2,3-deossigenasi, indotta dal cortisolo.

In conclusione, questi risultati sono da considerarsi come un primo step per indirizzare, in modo più corretto, ulteriori ricerche sull'effetto della manipolazione della dieta culle concentrazioni ematiche e cerebrali di serotonina.

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Separation related disorders in Poodles

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Abstract: Aim of this study was to verify the incidence of Separation Related Disorder (SRD) in Miniature and Toy Poodles. A questionnaire was used, divided into three sections: the first concerning the personal data of the owner, the second the dog (age, sex, castration and date of acquisition) and the last one intended to analyze the behavior of the animal.

Questionnaires received were 227, of which 124 regarding males (17 castrated) and 103 females (34 neutered). Among the owners, 44.49% of them believed their animals (n = 101) experience SRD. The statistical analysis revealed no significant differences between the two sexes of animals, with an incidence of separation problems of 41.7% among the males and 46.8% among the females (X2 = 0.576; n.s.).

The most common behaviors encountered during periods of loneliness by animals showing SRD were vocalizations (76.24%), destruction (28.71%), inappropriate eliminations (16.83%), loss of appetite (12.87%) and lastly excessive salivation (11.88%).

Vocalizations (♀ 86.0% vs ♂ 69.0%; X2 = 3.977; p = 0.047) and excessive salivation (♀ 17.2% vs ♂ 4.7%; X2 = 3.793; p = 0.05) were more statistically presented by females than males.

These data represent a first analysis of the incidence of Separation Related Disorder in Miniature and Toy Poodle. A great percentage of owners said that their animals show discomfort when being alone at home: this data permit to suppose that this breed is particularly predisposed to live the periods of loneliness with difficulty.

Key Words: Poodle; separation related problems

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Introduction

The companion dogs, as every social species, form bonds with conspecifics (Mariti et al., 2014), but also strong social relationships with humans (Mariti et al., 2013; 2017; 2018). In fact, they have evolved a capacity for attachment to their owner that is functionally analogous to human infants' attachment towards their mother (Ainsworth, 1969).

The most relevant characteristics of attachment are 1) contact-maintenance with the attachment figure, 2) separation distress in the absence of the attachment figure, and 3) the secure-base effect, i.e., the activation of exploratory and play behavior in the presence of the attachment figure (the attachment figure represents a base from which to explore the world) (Bowlby, 1969).

Although most dogs experience mild levels of stress upon separation from their attachment figure, some dogs show higher than normal sensitivity to separation and exhibit severe behavioral signs when left alone, generically defined as Separation Related Disorder (SRD), (Konok et al., 2019).

SRD is characterized by a high level of anxiety occurring in the absence or perceived absence of the owner (attachment figure) (Horwitz, 2000). It manifests in many behavioral and physiological signs, such as destructive behavior displayed in the home, excessive vocalization and

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inappropriate elimination (urination/defecation). Further signs include autonomic reactions (hypersalivation or hyperventilation), increased and repetitive motor activity (e.g. pacing, circling), other repetitive behaviors (e.g. over-grooming or self-mutilation), behavioral signs of depression (withdrawal, inactivity or inappetence), gastrointestinal signs (e.g. vomiting, diarrhoea) or escape behavior that can result in self-trauma (Appleby & Pluijmakers, 2004).

Poodles are a group of formal dog breeds, the Standard, Medium, Miniature and Toy Poodle. This breed descends from the Barbet of which it has conserved many characteristics. In 1743, it was called the "caniche": the female of the barbet in French. Thereafter the Barbet and the Caniche (Poodle) were gradually separated (FCI, 2016). The Poodle became very popular as a companion dog because of its friendly, joyful and loyal character and because of its four sizes and different colours which everyone can choose according to preference (FCI, 2016). The most popular varieties today are Miniature (over 28 cm up to 35 cm) and Toy Poodles (24 cm up to 28 cm).

Aim of this study was to verify the incidence of SRD in Miniature and Toy Poodles.

Material and methods

For this research was used a questionnaire divided into three sections: the first concerning the personal data of the owner, the second about the dog data (age, sex, castration and date of acquisition) and the last one intended to analyze the behavior of the animal.

The module, created through Google Forms, was posted in four different closed groups of owners of Miniature and Toy Poodles on the Facebook social network.

The statistical analysis of the data was carried out with the Chi-square test.

Results

Questionnaires received were 227, of which 124 were on male dogs (17 castrated) and 103 on female dogs (34 neutered).

The average age of the owners was 45.5 ± 11.3 years, mostly female (95.6%). The age of dog adoption, reported by the owners, was 4.4 ± 9.0 months. Fifty one percent of the poodles was purchased from professional breeders, 25% from private ones, while the remaining 24% had different origins.

As for the time spent in loneliness during the day, 58% of dogs remains alone from 1 to 4 hours a day, 23% from 4 to 8 hours and 13% less than an hour a day.

The analysis of the places where dogs are left during the moments of solitude reveals that the house is the favorite place of the owners (78%) when they must leave the dog alone. The home garden is preferred by 7% of the owners and the dog nursery by 4%.

Among the owners, 44.49% of them believed their animals (n = 101) experience SRD. The statistical analysis revealed no significant differences between the two sexes of animals, with an incidence of separation problems of 41.7% among the males and 46.8% among the females (X^2 = 0.576; n.s.)

The most common behaviors encountered during periods of loneliness by animals showing SRD were vocalizations (76.24%), destruction (28.71%), inappropriate eliminations (16.83%), loss of appetite (12.87%) and lastly excessive salivation (11.88%).

Vocalizations (♀ 86.0% vs ♂ 69.0%; X^2 = 3.977; p = 0.047) and excessive salivation (♀ 17.2% vs ♂ 4.7%; X^2 = 3.793; p = 0.05) were more statistically presented by females than males. There were no significant differences between males and females about destructive behaviors (♂ 32.6% vs ♀ 25.9%; X^2 = 0.541; n.s.), inappropriate eliminations (♂ 16.3% vs ♀ 17.2%; X^2 = 0.016; n.s.) and inappetence (♂ 14.00% vs ♀ 12.1%; X^2 = 0.078; n.s.).

Destructions were directed, above all, towards owners' objects like clothes and slippers (43%); 33% of dogs destroyed their toys, walls, doors, sofas, curtains and armchairs and finally 24% of dogs the chairs.

About the presence or not of other animals in the family, 104 Poodles were the only animals present,while 42 lived with another dog, 26 with a cat and 15 with both a dog and a cat.

The incidence of animals with separation problems was greater among dogs cohabiting with a cat (15.8% vs 7.9%; $p = 0.68$), showing a near-significant trend.

Discussion

These data represent a first analysis of the incidence of SRD in Miniature and Toy Poodle. A great percentage of owners (44%) said that their animals show discomfort when being alone at home, this data permit to suppose that this breed is particularly predisposed to live the periods of loneliness with difficulty. Although genetic predispositions cannot be excluded, it is clear from previous studies that the behavior of the dogs can be influenced by the amount of maternal care it has received (Guardini et al., 2015; 2016, 2017) and by social stimulations, also of a heterospecific nature, in the first period of life (Gazzano et al., 2008a) as well as the correct management of its behavior by the owners (Gazzano et al., 2008b).

Excessive barking in left alone dogs is traditionally considered to be one of the main signs of SRD (Flannigan & Dodman, 2001) in according with the results of this study. It is interesting to note that females display more vocalizations and salivation than males.

Another result that deserves attention is the greater presence of SRD in dogs cohabiting with a cat, with a value tending to significance: further research will be needed to clarify this aspect.

In conclusion, these first data seem to make plausible the hypothesis of a great incidence of SRD in Miniature and Toy Poodles. Further studies, on a larger sample and with a balanced percentage between owners of both sexes, will make possible to clarify the results of this pilot study.

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Problemi da separazione nel cane di razza Barbone

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Sintesi

Lo scopo di questo studio è stato quello di verificare l'incidenza di Problemi da Separazione (SRD) nei cani di razza Barbone, Miniature e Toy. A tale scopo è stato utilizzato un questionario, diviso in tre sezioni: la prima sezione riguardava i dati anagrafici del proprietario, la seconda riguardava il cane (età, sesso, eventuale castrazione, data di acquisizione) e l'ultima intendeva analizzare il comportamento degli animali.

Sono stati ricevuti 227 questionari, di cui 124 riguardanti Barboni maschi (17 castrati) e 103 femmine (34 sterilizzate).

Il 44,49% dei proprietari ritiene che il loro animali (n=101) manifestino segni di SRD. L'analisi statistica non ha rivelato nessuna differenza tra i due sessi di animali, con un'incidenza di SRD del 41,7% tra i maschi e del 46,8% tra le femmine ($X^2 = 0,576$; n.s.).

Gli animali che mostravano segni di SRD presentavano soprattutto vocalizzazioni (76,24%), distruzioni (28,71%), eliminazioni inappropriate (16,83%), inappetenza (12,87%) ed infine scialorrea (11,88%).

Le vocalizzazioni (♀ 86,0% vs ♂ 69,0%; $X^2 = 3,977$; $p = 0,047$) e la scialorrea (♀ 17,2% vs ♂ 4,7%; $X^2 = 3,793$; $p = 0,05$) erano manifestate maggiormente dalle femmine rispetto ai maschi.

Questi dati rappresentano una prima analisi dell'incidenza di SRD nei cani di razza Barbone Miniature e Toy. Un'elevata percentuale di proprietari ha affermato che i propri animali manifestano segni di disagio quando lasciati soli a casa; questi dati permettono di supporre che questa razza sia particolarmente predisposta a vivere i periodi di solitudine con difficoltà.

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Management of specific fears and anxiety in the behavioral medicine of companion animals: punctual use of psychoactive medications

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Abstract: A growing body of research has recently focused on the use of psychoactive medication for the short-term management of specific fear and anxiety in pet cats and dogs, i.e. triggered by well-identified and predictable stimuli. Such medications are used short-term and administered as needed to prevent the symptoms of fear and anxiety associated with specific situations. Noticeably, in the last few years two medications have been approved in the US and Europe for the treatment of noise fear in dogs. Furthermore, literature from the past decades provides evidence of the anxiolytic effect following the administration of several other medications used in various situations such as travelling and veterinary examinations. This review provides a summary of the most appropriate medications for punctual use in case of specific fear and anxiety in cats and dogs. The authors recommend the use of psychoactive medications providing a clear anxiolytic effect, especially in association with environmental management and humane behavioral procedures, such systematic desensitisation and counterconditioning. Combination treatment with non-psychoactive medications is also discussed.

Key Words: anxiety, psychopharmacology, acepromazine, dog, cat.

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Introduction

The recent years have seen a growing body of research on the application of psychoactive medication for the temporary management of fear and anxiety in pet cats and dogs. These medications are usually indicated for short term, punctual use, i.e. to alleviate the symptoms of fear and anxiety associated with specific situations. Historically, acepromazine (ACP) has been for a long time the sole psychoactive drug, originally approved in several countries "as an aid to control intractable animals" and "as an antiemetic agent to control vomiting due to motion sickness in dogs and cats" (Plumb, 2018). With time, acepromazine has become also commonly used in various situations that caused increased reactivity in the animal or were associated with symptoms of anxiety and fear; such as the exposure to thunderstorms and fireworks. Despite such common use of this medication, acepromazine is a phenothiazine derivative, which does not alleviate the animal's emotional state of fear and/or anxiety (Sümegi et al., 2014); in fact, the tranquilliser effect is rather dependent on motor inhibition mechanisms. The mechanism of action of acepromazine relies on the blockage of dopaminergic receptors situated in the basal ganglia and in the limbic system, whose activation is associated with CNS depression, sedation, and motor incoordination.

Nowadays, authors agree that phenothiazines are not the drug of choice for the treatment fearful or phobic behaviors (long or short term), because they have poor anxiolytic activity, they induce marked sedation and even heighten the animals' sensitivity to stimuli, while they have no means to escape due to the motor inhibition effects. This combination of effects induces an aversive experience that might lead to worsening of the behavior problems by increasing the sensitivity to the

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triggering stimuli (sensitization) (Clough, 1982). According to some authors dogs may become more reactive to noise after treatment with ACP (Landsberg, 2003; Overall, 1997; Overall, 2002). On the contrary, dissociative agents, such as acepromazine, reduce the animal's general ability to process and elaborate environmental stimuli, which is likely to increase rather than decrease the animal's anxious response to the triggering stimulus (Overall, 2013). Furthermore, the duration and degree of sedation may vary among individuals and might be breed dependent (Thompson, 1998). It should also be noted that paradoxical reactions and aggressive dis-inhibition have been observed after acepromazine administration, which poses a risk for humans and other animals that are in contact with the treated individual (Crowell-Davis,Murray & de Souza Dantas, 2019).

Overall, it is therefore clear that phenothiazines are not appropriate medications for the treatment of anxious, fearful, or phobic animals because they have poor anxiolytic effect and produce marked sedation and potential sensitisation to the triggering stimuli (Thompson, 1998). The use of acepromazine in dogs and cats that show signs of distress during specific situations (e.g. car transport, sudden noises) not only is not related to long term improvement of the emotional and behavioral signs, but it might even further compromise the welfare of the animal. For these reasons the authors agree that the use of ACP should be avoided, especially as a sole or preventive medication, for the treatment of animals that show signs of distress associated with specific events, such as travel, exposure to loud noises or during veterinary examinations. Likewise, the authors disagree with the use of any strategy based on coercive methods (e.g. physical punishment) or flooding (i.e. the continuous exposure of an animal to an aversive stimulus). The authors recommend the use of psychoactive medications with a clear anxiolytic effect, especially in association with environmental management and humane behavioral procedures, such systematic desensitisation and counterconditioning.

General overview of the behavioral signs related to specific fear and anxiety in cats and dogs

Cats and dogs manifest their discomfort in situations such as travel by car, airplane, or train, or during the exposure to loud noises through behaviors that are commonly associated with anxiety and fear of various intensity, depending on the individual. Such behaviors include vocalizations, shaking, mydriasis, polypnea, tachycardia, drooling, hyperactivity and pacing, escape attempts, searching for hiding places, inappropriate urination and/or defecation (Anon, 1998; Beaver 1999; Bergeron, 2002; Blackwell et al., 2005; Bower and Youngs 1994; Crowell-Davis, 2019; Dale et al., 2010; O'Farrell 1992; Mariti et al., 2012; Mugford, 1991; Schwartz, 1997; Storengen & Lingaas, 2015).

Car travel

Owners often travel by car with their pets for short journeys (e.g. few minutes to reach a park) or long journeys (e.g. long hours to arrive to a holiday destination). Various behavioral responses to distress in these situations have been described in cats and dogs: some manifest signs of motion sickness (nausea, vomiting), others manifest signs of distress or simple over excitement in anticipation of the event following the travel or the journey itself (Anon, 1998; Beaver, 1999; Bower & Youngs, 1994; O'Farrell, 1992; Mugford, 1991; Schwartz, 1997).

Airplane travel

Acepromazine has been widely prescribed in occasion of airplane journeys; however, evidence indicates that stress signs during the journey do not change in dogs treated with ACP (Bergeron,

2002). Furthermore, the International Air Transport Association recommend to not use any drug to tranquilise pets prior to transport, due to health and safety reasons for the animal itself.

Noise sensitivity

Several studies indicate that nearly half of the pet dog population may be fearful or anxious when exposed to various of auditory stimuli, such as storms and fireworks (Blackwell et al., 2005; Crowell-Davis, 2019; Dale et al., 2010; Mariti et al., 2012; Storengen & Lingaas, 2015). It is however possible that these figures underestimate the real frequency of occurrence of the issue. Furthermore, the problem poses a considerable risk for the animals, which may become seriously injured or lost while attempting to escape from the triggering stimuli.

Drugs indicated for punctual use in case of specific fear and anxiety in cats and dogs

Given the considerations above, veterinary practitioners should consider other alternatives to the use of ACP to provide short-term support (i.e. punctual use) for anxious, fearful and phobic cats and dogs in the case of specific triggering situations. All the dosages and formulations discussed below are for oral administration (i.e. *per os*) except when differently specified.

Overall, the successful treatment of fearful and anxious pets aims to: a) relieve the distress and the negative emotional state during the triggering situation (stressful event) and consequently improving the behavioral responses of the animal; b) support a long term behavioral modification programme that aims to modify the emotional response to the triggering situation (these include desensitisation and counterconditioning).

Labelled products

Dexmedetomidine

Dexmedetomidine oro-transmucosal gel (Sileo®) is a drug approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) to reduce acute anxiety and fear associated with noise aversion in dogs. The active ingredient is an alpha-2 adrenoceptor agonist which reduces the signs of acute anxiety and fear associated with noise in dogs when administered at the dose of 125 micrograms/m2 - please note that the formulation requires oro-transmucosal absorption (Dean, 2017; Korpivaara et al., 2017). Clinical studies have found the drug to be effective to reduce anxiety during travel (Landsberg et al., 2018) and veterinary examinations (Jonckheer-Sheehy & Zaal, 2018). Owners must be informed about the correct administration of the medication, i.e. in the buccal area. The gel is not for oral administration and should not be swallowed; the effects following oral administration of this formulation are not predictable.

The anxiolytic effect of dexmedetomidine is linked to the alpha-2 adrenoceptor agonist action in the *Locus coeruleus* (Aantaa et al., 1993; Murrell & Hellebrekers, 2005). Sileo® should be administered 30-60 minutes before the triggering event; the effect lasts for 2-3 hours. The administration can be repeated up to 5 times in 24 hours, which should be taken into account for long travel or during hospitalization. There is no information in the literature about the use of Sileo® in cats.

No information is available on the interactions between Sileo® and other drugs. However, the use of Sileo® in combination with sympathomimetic amines is highly discouraged. The use of the product during pregnancy, cardiovascular and systemic pathologies, and in senior pets is not recommended (BSAVA, 2017).

Imepitoin

Imepitoin (Pexion®) has been recently approved by the EMA for the reduction of anxiety and fear associated with noise phobia in dogs. Imepitoin has an anxiolytic effect, due to the partial activation of the receptors for the neurotransmitter GABA-A and weak blocking effect on calcium channels (Denenberg & Bräm Dubé, 2018; Engel et al., 2018). The anxiolytic effect of Pexion® starts after a few days of administration, therefore some authors suggest that the treatment should start a few days before the triggering event (Denenberg & Bräm Dubé, 2018; McPeake et al., 2017).

A series of case studies has found that, in dogs, Pexion®, administered at the dose of 10-30 mg/kg BID, reduces signs of distress and induces a positive emotional state in case of fear and anxiety related problems (McPeake et al., 2017). The results were further confirmed by a placebo-controlled clinical trial indicating that Pexion®, administered at the dose of 30 mg/kg BID starting 2 consecutive days before the expected triggering noise event, significantly reduces the behavioral signs of fear and anxiety, when compared to a placebo (Engel et al., 2019). In the literature, the use in cats is suggested at a dose of 10-30 mg/kg BID, starting 5-10 days before the triggering event (Denenberg & Bräm Dubé, 2018). It should be noted that the use of Pexion® for cats is not yet approved by the EMA or the FDA.

Overall, it is recommended for the treatment to start at the lowest dose and titrate upward if needed until the effect has been reached (Denenberg & Bräm Dubé, 2018; McPeake et al., 2017). In case of side effects, doses should be reduced (McPeake et al., 2017).

Off-label products

In Europe, a number of psychoactive medications are used off-label based on evidence in the literature and may be prescribed taking into account the principles of the Cascade. The Cascade is a risk based decision-tree aiming veterinary surgeons in the decision process to select the product to use when there is no authorised veterinary medicine available for a specific condition or species. In certain countries (e.g. Italy) the use off-label may be approved based on scientific evidence where a given product is available but proves not to be effective in a certain individual. Without the Cascade, veterinary surgeons could only prescribe veterinary medicines that are authorised for a given species and for a given condition.

Benzodiazepines

Various Benzodiazepines are reported in literature to be effective in case of anxiety and fear in small animals: for example, Lorazepam 0.02–0.1 mg/kg PO every 8-12 hours. Benzodiazepines increase the effect of GABA on the GABA-A receptors, with anxiolytic, relaxing, antiepileptic, and muscle-relaxing effects (Dodman & Shuster, 1994). Owners should be warned that benzodiazepines might have dis-inhibitory effect, therefore they may disinhibit aggressive behavior (Crowell-Davis,Murray & de Souza Dantas, 2019).

Gabapentin

Gabapentin has been recently discussed in the literature as a safe and effective treatment for cats to help reduce stress during transportation and veterinary examination, and for trap-and-release procedures in feline colonies (van Haaften et al., 2017; Pankratz et al., 2017).

The anxiolytic mechanism of Gabapentin is not well understood, although it is suspected to be related to the effect on calcium channels in the neural tissue (Cheng & Chiou, 2006; Davies et al., 2007). Additionally, Gabapenting is an effective medication for the management of neuropathic pain both in cats and dogs (e.g. Siao et al., 2010; Moore, 2016).

In cats, Gabapentin has been used to reduce stress and aggression and increase compliance dur-

ing transportation and veterinary examination, when administered in a single dose of 50-100 mg / animal, 90-120 minutes prior to placing the cat into a carrier. In smaller cats Gabapentin might be administered at the lowest dose in order to avoid sedative effect (van Haaften et al., 2017; Pankratz et al., 2017).

Suggested doses for dogs range from 2-5 mg/Kg BID to 10-20 mg/Kg BID or TID (Overall, 2013). The effect of Gabapentin lasts for about 8 hours and should not be repeated more than 3 times within 24 hours. The authors have found the 10-20 mg/Kg dosage to be effective, especially in combination with other medications (e.g. Trazodone).

Trazodone

Trazodone is classified as a Serotonin Antagonist and Reuptake Inhibitor (SARI) and in humans is used as antidepressant, anxiolytic, and for the treatment of OCDs.

This psychoactive drug has a complex pharmacologic mechanism, which includes and antagonist action on the 2A serotonergic receptors and the post-synaptic reuptake of serotonin (Stahl, 2009). The use of Trazodone has often been reported in the literature for its anxiolytic effect in dogs (e.g. Gruen & Sherman, 2008; Gruen et al., 2017). The medication has also been used for the treatment of nocturnal activity both in cats and dogs, especially when associated with anxiety, due to its mild sedative effects (Gruen et al., 2014; Orlando et al., 2016).

The suggested dosage for Trazodone in dogs ranges between 1.7 and 9.5 mg/kg in a single dose to be administered 90 minutes prior to the stressful event. The administration may be repeated for a maximum of 3 administrations in 24 hours (Overall, 2013).

In cats, Trazodone has been suggested in the literature to reduce the symptoms of stress during transportation and veterinary examinations (Stevens et al., 2017). The suggested dose is 50 mg/cat to be administered 60-90 minutes before the triggering event. It has been reported that in a few cases trazodone had a dis-inhibitory effect, which might lead to increased aggressive response (Gruen & Sherman, 2008). Therefore, the use of Trazodone in animals with a history of aggressive behavior should to be considered cautiously.

Combined use of psychoactive and non-psychoactive medication

Anxiety and fear-related responses to certain stimuli might be related to physical causes of distress for the animal (Reaney et al., 2017). These might be unrelated with the triggering situation, such as acute and chronic pain (Affenzeller et al., 2017; Barcelos et al., 2015; Camps et al., 2012; Lopes Fagundes et al., 2018), or they might be event-related consequences, such as motion sickness. Therefore, it is recommended that pain-relief or other relevant medication is used in combination with psychoactive drugs, in order to alleviate the symptoms associated with the triggering situations.

For example, recent evidence in the literature lead to the recommendation that pets that presenting with noise sensitivities (anxious, fearful, phobic or over-reactive responses to noises) should be assessed and treated for pain related problems (Lopes Fagundes et al., 2018). The authors would like to stress that adequate pain management (especially in the case of musculoskeletal pain) should also be a key aspect of the long and short-term medical treatment of pets showing signs of distress associated to travel, due to the continuous solicitations on the joints and musculoskeletal system associated with transport.

Another aspect may lead to distress and consequent sensitisation, especially during travel and transportation, may be motion sickness. The management of gastrointestinal effects caused by motion sickness is associated with a less stressful experience for the pet during travelling. Maropitant citrate, an NK1 antagonist is indicated for the prevention and treatment of acute vomit-

ing during travel (Benchaoui et al., 2007; Hickman et al., 2008). The medication does not induce behavioral inhibition that is associated to other drugs such as ACP. In order to prevent motion sickness, Maropitant is administered at a minimum dose of 8 mg/kg once 1 hours prior to travel (Benchaoui et al., 2007). It should be noted that the dosage needs to be adjusted in cardiopathic animals since Maropitant affects calcium channels.

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Trattamento della paura e dell'ansia in medicina comportamentale: corretto uso di psicofarmaci

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Sintesi

Numerose ricerche sono state effettuate sull'uso di psicofarmaci per il trattamento a breve termine di casi specifici di paura ed ansia nel cane e nel gatto, ovvero scatenati da stimoli ben evidenti e prevedibili.

Questi farmaci sono utilizzati per breve tempo e somministrati al bisogno per prevenire i sintomi della paura e dell'ansia associate a particolari situazioni.

Negli ultimi anni due farmaci sono stati approvati negli Stati Uniti ed in Europa. La letteratura degli ultimi dieci anni fornisce evidenze di effetti ansiolitici conseguenti alla somministrazione di altri farmaci usati in varie situazioni come ad esempio i viaggi e le visite veterinarie.

Questa review elenca i farmaci più appropriati per un uso corretto in casi specifici di paura ed ansia in cani e gatti.

Gli autori raccomandano l'uso di psicofarmaci che forniscano un chiaro effetto ansiolitico, specialmente in associazione con gestione dell'ambiente di vita e delle relazioni con l'uomo, come la desensibilizzazione sistematica ed il controcondizionamento. È inoltre presentato l'uso combinato con altri farmaci non psicoattivi.

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A case of dysthymia in a Yorkshire terrier

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Abstract: A 16 years old spayed female Yorkshire terrier weighting 4.1 kg, was evaluated because, in recent months, she started barking, crying and howling.

Considering the dog history and behavioral signs, it was considered possible that the aggressiveness and the crying could be related to painful arthritis.

The Age-Related Cognitive and Affective Disorders (ARCAD) scale was provided to the clients and a score of 27 was obtained, compatible with dysthymia. Therefore, also considering the main behavioral symptoms, dysthymia was considered the most probable diagnosis and the dog was treated consequently.

To relief the dog in the moment of acute outbreaks of arthrosis' pain, Meloxicam (Inflacam®) 0.2 mg/kg was prescribed to be given the first day, followed by 0.1 mg/kg q24h for six days, and Cartimax® mini, (sid, sine die).

Concerning gastrointestinal symptoms, Canikur® (sid for ten days), Phosphaluvet® (1.5 ml, 3 times per day, for 3 days) and Maropitant (Cerenia®; 2 mg/kg, in pills for 3 days after a first injection 1 mg/kg), were added at the original therapy.

A treatment with Selegiline (Selgian®) 0.5 mg/kg was prescribed, as advised by Landsberg (2013). The owners were warned that the treatment, if effective, should be maintained during the whole life of the dog.

The dog was brought to visit almost once a week. The diarrhea and vomit stopped. Dermatological problems did not come back. Nonetheless, behavioral improvements were not shocking.

Considering the multifactorial nature of the process, a holistic approach to treatment is necessary, and each individual component needs to be identified and solved. Furthermore, owners of senior pets should be clearly instructed by the veterinarian regarding the correct behavior to have. On the other hand, they should work closely with the veterinarian to report any behavioral change as soon as it arises.

Key Words: aged dog, dysthymia, selegiline

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Presentation

A 16 years old spayed female Yorkshire terrier weighting 4.1 kg, was evaluated because, in recent months, around 10 P.M., she started barking, crying and howling. She looked lost and confused, and she often run into or got stuck into objects, doors, walls and corners. Overall, she looked restless and anxious. When the crisis began, the owners were not able to approach nor comfort the dog in any way, as she tried to bite them violently. She also showed vomit and diarrhea that was not controlled by previous treatments.

History and presenting signs

The dog lived in Corse with another Yorkshire terrier (11-year-old spayed male); the owners were a middle-aged couple. They lived in a medium-sized house with a large garden. The dog

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had always lived there and both dogs had access to all the rooms of the house, in any moment. There was no history of fighting between the two dogs nor of other behavioral problems expressed by the female dog before, according to the owners. In particular, the dog never used to bite other animals or people. The owners said she was the exact opposite of what she used to be. Nonetheless, they claimed never having punished her, physically or verbally.

Both dogs had access to the garden when they asked for it and they were regularly taken for a walk on a leash at least once per day.

Both owners worked, nonetheless they managed to spend a good amount of time with their dogs, at the house or outside. In any case, the two dogs were used to stay quietly at home when they were left alone. Overall, nothing had changed in the house in the last few months.

The dog was originally taken to visit because of persistent diarrhea and vomiting. Sometimes she vomited right after eating her meal, sometimes after some hours. Defecation was altered in terms of consistence and texture, but not frequency. The dog was treated by another veterinarian of the same clinic with a product based on Spiramycin and Metronidazole (Stomorgyl® 12.5 mg/kg sid for 10 days), Spasmoglucinol® (4 mg/kg bid for three days) and Ultradiar® (one pill per day, for 7 days). She had an history of dermatological issues that seemed to be solved.

After one week of treatment, the symptoms had not satisfactorily changed.

Urination behavior was normal, as well as drinking and feeding. Except for the vomiting, her weight was stable and her appetite unchanged.

During the visit, the owners referred that the previous veterinarian had ascribed the behavioral problem to a diminished vision. Therefore, in the evening, the owners turned on almost every light in the house in order to help the dog, but no changing in her behavior was achieved.

Even if this was not the first cause for consultation, they seemed very concerned and almost scared of dog behavior, because when she started crying in the evening, they felt she really was suffering. The dog seemed less affectionate towards the owners, but she rarely bit them, whereas in the evening she was unapproachable.

Physical Examination Findings and Laboratory Results

Physical examination showed a diminished vision, due to a problem of bilateral cataract, and diminished hearing.

The palpation revealed a lumbar and hind limbs pain, compatible with a problem of arthrosis and it was confirmed by the evidence of parakeet beak on various lumbar vertebrae, highlighted with a RX. A slight abdominal pain was also remarked, as well as bloating.

A blood sample was taken and laboratory analysis (blood biochemical and hematologic assessment, as well as thyroid testing) were performed and the results were unremarkable.

A EMR was recommended to exclude brain neoplasia, but the owners were reluctant.

A fecal sample was taken but no parasite was found, consistently with the owners regularly deworming their dogs, and the giardia test was negative. An echography was performed to exclude the presence of foreign bodies.

The dogs were always brought together to the consultation. During the visit, the female dog walks around panting, vocalizing and she almost never sat down. She repeatedly licked her lips, yawned and snooze. She did not appreciate to be touched, exposing her teeth while staring at the veterinarian's hands. These behaviors were interpreted as a state of anxiety (Landsberg, 2013), rather than simple fear of the consultation, particularly because the owners said that she never behaved in that way and veterinarians were usually happy to visit her because she was very calm.

Diagnosis

Considering the dog history and behavioral signs, it was considered possible that the aggressiveness and the crying could be related to painful arthritis. Back pain could also be responsible for the gastrointestinal symptoms and bloating. The co-existence of different medical conditions, such as gastrointestinal disturbances and physical pain, might lower the level of one's tolerance, leading to an increase in frequency or severity of anxious or phobic behaviors, possibly leading to a subsequent increase in gastrointestinal disturbances, like in a vicious circle.

Dermatological problems, that had been simply treated as such, could have partially been a consequence of an anxious state.

In any case, health problems generally lower the tolerance threshold of an animal, who possibly had already begun performing avoidance behaviors, without the owners realizing it. Furthermore, even if medical problems can be resolved, the behavior problem might persist if the pet has learned that aggression can successfully remove anxious or annoying stimuli such as unwanted or painful contact.

The suddenness in the appearing of the symptoms allowed to consider the presence of a brain neoplasia, although it was difficult to discern if the symptoms truly had a sudden outbreak or if they had been so subtle that the owners had not noticed them.

However, due to the presence of disorientation (D), altered interaction with people and other animals (I), altered sleep-wake cycles and altered activity (S), partial activity level change (A) although the absence of house soiling (H), behavioral problems related to aging cannot be excluded, specifically Cognitive Dysfunction Syndrome (CDS) (Chapagain, 2018; Mongillo, 2010).

Nonetheless, the French approach according to Pageat was also considered (Pageat, 1998). Due to the marked temporal disorientation with the dog being more active in the evening/night, alternated with almost normal periods during the day, confusional syndrome (also referred to as canine cognitive disorder) of old dogs is taken into account. Dysthymia is also considered, as the most typical characteristic of this condition is the loss of body length and size, associated with aggressive response triggered by an attempt of external help, growling and whining (Pageat 1995; Pageat 2001).

Involutive depression was regarded as less probable due to the absence of enuresis, encopresis, absence of house soiling and of resumed oral exploration of the environment.

A connection between the digestive symptoms, stress and anxiety associated with aging-related neurologic changes was hypothesized (Gualtieri, 2010; Reiwald et al., 2013; Vermeire et al., 2009).

A French translation of the ARCAD scale as reported by Landsberg et al., 2013, proposed by Pageat (2001), was provided to the clients and a score of 27 (14 affective or emotional parameters + 13 cognitive parameters) was obtained.

According to this scale, a score between 22 and 30 is compatible with dysthymia. Therefore, also considering the main behavioral symptoms, dysthymia was considered the most probable diagnosis and the dog was treated consequently.

Treatment

Because the observed symptoms could be linked with a multiplicity of causes, the treatment involved a combination of environmental management, behavior modification, behavior medication and medical treatment of health problems.

To relief the dog in the moment of acute outbreaks of arthrosis' pain, Meloxicam (Inflacam®) 0.2 mg/kg was prescribed to be given the first day, followed by 0.1 mg/kg q24h for six days, and Cartimax® mini, (sid, sine die).

Concerning gastrointestinal symptoms, Canikur® (sid for ten days), Phosphaluvet® (1.5 ml, 3 times per day, for 3 days) and Maropitant (Cerenia®; 2 mg/kg, in pills for 3 days after a first injection 1 mg/kg), were added at the original therapy.

A treatment with Selegiline (Selgian®) 0.5 mg/kg was prescribed, as advised by Landsberg (2013). The owners were warned that the treatment, if effective, should be maintained during the whole life of the dog.

Selegiline is a selective and irreversible inhibitor of monoamine oxidase B (MAOB) (Milgram et al., 1993; Gerlach et al., 1994). Enhancement of dopamine and perhaps other catecholamines in the cortex and hippocampus is presumed to be an important factor in clinical improvement in dogs (Knoll, 1998). Selegiline metabolites, l-amphetamine, and l-methamphetamine may also enhance cognitive function. Furthermore, Selegiline may contribute to a decrease in free radical load in the brain, by directly scavenging free radicals and enhancing scavenging enzymes, such as catalase and superoxide dismutase (SOD), the latter of which is increased in dogs on selegiline therapy (Carillo et al., 1994). Overall, Seleginine is believed to have neuroprotective effects (Heinonen and Lammintausta, 1991).

A hypoallergenic diet was adopted. Senilife® was recommended to slow down aging-related cerebral modification (Osella et al., 2006; Lant et al., 2013; Colangeli et al., 2005) and Canergy® (propentofylline) was recommended to improve cerebral vascularity and oxygenation.

The owner was instructed to increase the environmental enrichment through increased social interaction, when the dog was consensual, without forcing her.

It was also recommended to the owner to introduce new toys in the environment, increase the time spent walking the dog outside (in a way that was compatible with her musculoskeletal pain) during the day, in order for her to be more tired in the evening and adapt her safe zone in a quiet corner of the house with a not painful or stressful access for her. Any change should be introduced slowly to reduce potential stress and pre-existing sources of stress and anxiety should be identified and avoided. Owners were also encouraged to maintain a stable and predictable environment and schedule, in order to increase predictability and decrease uncertainty and, consequently, anxiety. For the same reason, and to teach the dog new behavioral responses, the owners were advised to teach the "sit-down" command, asking the dog to assume a quiet posture before every interaction with them.

The use of DAP (dog appeasing pheromone) in the form of ADAPTIL® collar was also recommended in order to reduce anxiety and stress (Landsberg et al., 2015; Pageat et al., 2003).

Follow up

The dog was brought to visit almost once a week. The diarrhea and vomit stopped. Dermatological problems did not come back. Nonetheless, behavioral improvements were not shocking. Although the owners were warned that a response to Seleginine could take up to two months,, they seemed quite hopeless after less than a month and it was possible to assume that they had not given the therapy as recommended.

In any case, the owners were advised to continue the therapy as prescribed and to follow attentively every aspect of it, before applying changes to it.

Discussion and conclusion

As pets age, there are a number of health problems where a change in behavior is noticed as the first sign of illness (e.g. pain, sensory decline, neurological diseases...). On the other hand, aging is a multifactorial process that leads to a general deterioration in physical condition, such

as decreased cerebral perfusion (Peremans et al., 2002) and general tissue hypoxia, alteration in cell membranes, increased production of reactive oxygen species and decreased competence of the organism to clear them up, decreased response from the immune system etc. For these reasons, aged pets are less capable to maintain their homeostatic balance.

All these things combined could challenge the relationship between the dog and his owner. Furthermore, until recent times, the cognitive and emotional aspects of aging have been greatly overlooked and changing in behaviors have been dismissed as "old age", without little consideration to the fact that aging is a physiological process that doesn't necessarily involve diseases, discomfort and reduced quality of life.

Considering the multifactorial nature of the process, a holistic approach to treatment is necessary, and each individual component needs to be identified and solved. Furthermore, owners of senior pets should be clearly instructed by the veterinarian regarding the correct behavior to have. On the other hand, they should work closely with the veterinarian to report any behavioral change as soon as it arises. Only with a good collaboration between the two sides, good results can be achieved, appropriate diagnostic can be carried out and diseases and stressful/painful conditions of the animals can be avoided, thus improving their quality of life and, possibly, longevity.

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Un caso di distimia in un Yorkshire terrier

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Sintesi

Uno Yorkshire femmina, di 16 anni, castrata (4,1 Kg di peso) è stata portata in consulenza perché negli ultimi mesi abbaiava, si lamentava ed ululava.

Considerando la storia clinica del soggetto e i segni comportamentali, è possibile che l'aggressività e le vocalizzazioni possano essere correlate ad una forma dolorosa di artrite.

La scala ARCAD ha fornito uno score di 27, compatibile con una diagnosi di distimia. Per dare sollievo al cane in corso di episodi di dolore artrosico acuto è stato prescritto Meloxicam (Inflacam) (0,2 mg/kg il primo giorno, seguito da 0,1 mg/kg q24h per 6 giorni) e Cartimax mini, (sid, sine die).

Per quanto riguarda i sintomi gastroenterici, sono stati aggiunti alla terapia Canikur (sid per10 giorni), Phosphaluvet (1,5 ml, 3 volte al giorno per 3 giorni) e Maropitant (Cerenia; 2 mg/kg, in compresse per 3 giorni dopo una prima iniezione di 1 mg/kg).

È stato quindi prescritto un trattamento con Selegilina (Selgian) 0,5 mg/kg.

I proprietari furono avvisato che il trattamento farmacologico, se efficace, sarebbe dovuto continuare per tutta la vita dell'animale.

Il cane fu condotto ai controlli una volta alla settimana: la diarrea ed il vomito scomparvero ed i problemi dermatologici non si ripresentarono, tuttavia non ci furono progressi significativi dal punto di vista comportamentale.

In conclusione, considerando la natura multifattoriale del processo di invecchiamento, è necessario un approccio olistico al trattamento ed ogni aspetto individuale deve essere identificato e risolto. Inoltre, i proprietari di animali anziani dovrebbero ricevere dal proprio veterinario informazioni corrette riguardo al comportamento da avere. D'altra parte, essi dovrebbero collaborare strettamente con il veterinario per informarlo quanto prima di qualsiasi variazione del comportamento del proprio animale.