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Puppy food preferences are maintained in adulthood

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Abstract: Balanced diets that meet nutritional requirements for various life stages of animals are important to sustain health as dietary needs change with development. Dog owners want to offer a tasty and nutritionally balanced diet to their dogs. Dog food product developers strive to formulate such diets in a timely manner and that meet a dog's specific nutritional requirements in various life stages. Palatability assessments during product development can be expensive and time-consuming when evaluating specially designed foods for multiple breed types, dog sizes, and ages of dogs. Assessments of puppy food by puppies is especially expensive because a narrow window for food trials exists before they reach adulthood. Moreover, it is not practical or ideal for palatability assessment centers to continually acquire puppies when the dogs can only test as puppies for a fraction of their lifetime. Thus, we evaluated if preference trials of diets formulated specifically for small breed puppies could be assessed by small breed adults and yield similar results. We ran seven paired preference trials over 14 days with twenty dogs at ages 5-8 months old (i.e., puppyhood) and again at 14-17 months old (i.e., adulthood). In six of seven trials, dogs were consistent in their preference as adults and as puppies. While it is not recommended that dog owners feed their adult dog puppy food on a regular basis, the results suggest that pet food developers do not need to have constant access to puppy panels to evaluate palatability of puppy foods. Rather, adult dog panels could be a quicker, more practical, and more economical option to aid pet food developers in getting a product to market that puppies would likely enjoy.

Key Words: development; dog; food; palatability; preference; small breed;

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Introduction

Growing puppies should be fed a diet specifically formulated to meet their nutritional needs for normal development. Puppy foods are specially designed for their nutritional needs during growth, so feeding adult food will rob your puppy of important nutrients, such as higher levels of protein and fat (American Kennel Association, 2018).

Palatability is a critical component when formulating dog food in addition to essential nutrients, because a quality food is of little value if the dog will not eat it. "Palatability" describes how well a dog likes the taste, smell, and texture of a food (IAMS Pet Food, 2018). Premium dog food manufacturers spend considerable time conducting controlled feeding studies to determine the right combination of ingredients and processing techniques to produce a nutritious and palatable food (IAMS Pet Food, 2018). Palatability is vital during puppyhood to ensure food intake for normal growth and development.

Taste preferences of puppies may change as they age. A study of free-ranging dogs in India found that adult dogs showed a preference for meat, while puppies did not (Bhadra & Bhadra, 2014). Developmental changes in food preferences have been documented in a variety of other species from birds to humans. Fledged house sparrows abruptly switched from preferring insects

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to seeds (Mueller, 1986). Four to 24-month-old human infants showed a heighted acceptance for saline solution relative to water; however, by 31-60 months they tended to reject saline solution relative to water (Beauchamp et al., 1986).

We focused on small breed dogs because of the trend for increasing ownership of these dogs. Small breed ownership has increased 4% from 2012 to 2017 (Euromonitor, 2018), and pet food companies are responding to this by launching small breed puppy food lines. Blue Buffalo, Diamond, Eukanuba, Holistic, Iams, Nutro, Purina ProPlan, Royal Canin, and many other brands now produce small breed puppy foods, which are typically marketed for dogs less than one year of age. Small breed dogs, which are 9 kg (20 lbs.) or less when fully grown, usually transition to an adult formula when they are 9 to 12 months of age (IAMS Pet Food, 2018; Nestle Purina, 2018), whereas medium dogs transition between 12 and 14 months old, and large breeds (greater than 22.7 kg (50 lbs.) when fully grown) transition between 12 and 24 months of age.

Small breeds can be advantageous when assessing adult food preferences, because they reach adulthood sooner (Hawthorne et al., 2004). On the other hand, small breeds are puppies for less time than other breed sizes, which could be a disadvantage for assessing puppy food preferences long-term. Due to the relevance of having puppies assess puppy food, yet the narrow window of opportunity that small breeds are puppies, we address whether young adults are suitable to assess puppy food. If found to be suitable, costs of high-maintenance puppy panels at assessment centers could be reduced, a broader range of specially formulated foods can be assessed in a shorter amount of time and retaining quality assessment dog panels long term would be an economical benefit for assessment centers.

The current study assessed the stability of puppy food preferences from puppyhood (5-8 months of age) to young adulthood (14-17 months of age) for small breed dogs. The topic is of interest to both dog owners and dog food developers. Dog owners can be confident their puppy will enjoy a food despite palatability assessments by adult dogs, and food developers can be confident adult dogs are suitable, more practical, and more economical in assessments of puppy foods.

Material and methods

Subjects

Twenty small breed dogs were purchased at 8-11 weeks of age from a private breeder and raised at AFB International's Palatability Assessment Resource Center (PARC; Saint Charles, MO). Breeds and breed mixes included: Cavapoo, Cavalier, Poodle, Pekepoo, Yorkiepoo, Shihpoo, Teddy Bear, and Maltese. Dogs were spayed or neutered at 16 weeks of age. Fourteen were male and 10 were female; their adult weight ranged from 2.7 to 7.3 kg (mean = 5.9) (6 to 16 lbs.; mean = 13).

The dogs were group-housed but were individually kenneled during feeding trials. The amount of food a dog was allowed to consume in each trial was determined based on each dog's body condition score based on a 5-point scale (American Animal Hospital Association, 2010). This study was approved by the Institutional Animal Care and Use Committee (Protocol C1.001).

Food trials

The first round of feeding trials (puppy age; 5-8 months) was conducted in September 2017, and the second round (adult age; 14-17 months) in June 2018. Puppies were not assessed until 5 months of age because of training required to perform the pet food industry standard paired preference trial (described below).

Seven pairings of commercially available puppy foods were offered to dogs twice over 14 days

(Table 1) during each round. Food pairings and the order in which they were offered remained the same between feeding rounds. Foods were offered as paired preference trials in which a dog is offered two bowls during mealtime, each containing one of the two foods. For the second day of each pairing, placement of food bowls was alternated between the left and right side to account for potential side bias. Dogs had up to 20 minutes to finish their meal. Mealtime occurred once a day in the mornings.

Table 1. Feeding trial design where each pairing of fourteen commercially available foods (A-N) was offered for 2 days. The seven trials were conducted when dogs were 5-8 months of age and again at 14-17 months of age (n=20 dogs).

Day	Trial	Left Bowl Placement	Right Bowl Placement
1	1	A	B
2	1	B	A
3	2	C	D
4	2	D	C
5	3	E	F
6	3	F	E
7	4	G	H
8	4	H	G
9	5	I	J
10	5	J	I
11	6	K	L
12	6	L	K
13	7	M	N
14	7	N	M

Significance tests

Preference for one food over the other in each of the paired preference trials was determined by calculating the intake ratio. For each dog, the total amounts (in grams) of Foods A and B, for example, that were consumed over two days were calculated. The intake ratio was then calculated for each dog as the two-day total consumption of Food A divided by the two-day total consumption of both Foods A and B. Then, these two-day intake ratios were averaged over all dogs. The p-value is a measure of the statistical significance of the intake ratio. An intake ratio value that is statistically significant (< 0.05) indicates an average preference for that food. Intake ratios that are not significantly different are generally regarded as showing parity, or the lack of a preference for one food. The p-value is based on a two-sided statistical test.

Results

In six out of seven paired preference trials, dogs preferred the same puppy foods when they were puppy aged and when they were adult aged (Table 2). In two trials (Trials 6 and 7), parity preference was consistent across both rounds of feeding trials. Thus, despite no preference for one food over the other in these pairings, dogs were consistent in their no preference as puppies and as adults. In just one food pairing (Trial 5), dogs preferred one food over another as pup-

pies, but this preference did not persist as adults. These results support the suitability of young adult small dogs to assess preferences of puppy foods.

Table 2. Six of seven puppy food pairings yielded the same outcome when dogs were 5-8 months and 14-17 months of age (n=20 dogs).

Trial	Food Pairings	Dog Age					
		5-8 months			14-17 months		
		Preferred Food	Intake Ratios	p-value	Preferred Food	Intake Ratios	p-value
1	A vs B	A	98:2	<.001	A	83:17	<.001
2	C vs D	C	96:4	<.001	C	79:21	<.001
3	E vs F	F	23:77	<.001	F	29:71	<.001
4	G vs H	H	13:87	<.001	H	16:84	<.001
5	I vs J	J	30:70	<.05	NS[a]	40:60	>.1
6	K vs L	NS	53:47	>.1	NS	40:60	.096
7	M vs N	NS	56:44	>.1	NS	53:47	>.1

[a] NS indicates a non-significant result in which neither food was preferred.

Discussion

Our results support the suitability of young adult small breed dogs for assessing puppy food when determining food preferences of small breed puppies. In six of the seven paired food trials, the dogs showed the same preference pattern when they were young adults (14-17 months of age) as they displayed when they were puppies (5-8 months of age). Small dogs are only puppies for a short period of time, which creates a time constraint for food developers when fine-tuning formulas small breed puppies will enjoy. However, this constraint can be viewed as less of a concern because the majority of food preferences persisted in young adulthood.

We were unable to assess if the taste preferences of very young small breed puppies (2 to 4 months) would align with the preferences shown by 5-8-month-old puppies. It is possible small breed dogs may show different taste preferences from adults if assessed in early puppyhood. Development of puppies is a delicate period, deeply influenced by the mother (Guardini et al., 2015; 2016; 2017) and external stimuli (Gazzano et al., 2008) and categorized into four phases: neonatal, transitional, socialization and juvenile (Nott, 1992). Puppies in this study were assessed during the juvenile developmental period.

Previous studies have shown that diet experienced in puppyhood can influence the development of food preferences. Therefore, it was previously assumed that puppies may be required to assess palatability of puppy foods. Kuo (1967) found that puppies raised with limited exposure to foods of varying odor and taste during the first six months of life showed narrow food preferences. In contrast, puppies raised with exposure to a mix of foods with different flavors and textures showed greater acceptance of novel foods (Kuo, 1967). We observed food preferences were primarily maintained from the juvenile puppy period to young adulthood, which could be because all puppies in this study were raised on variety of puppy foods that varied in odor, taste, texture, and appearance. The practice of varying puppy foods is done so that puppies will sample and assess the palatability of a wide variety of dog food products as adults.

The assumption that palatability of puppy food diets needs to be assessed by puppy taste

testers may have been promoted by studies of developmental factors that influence flavor preference in neonatal puppies. Wells & Hepper (2006) demonstrated that flavors in the mother's milk and amniotic fluid influence neonatal flavor preferences of puppies. They also showed that postnatal, but not prenatal, exposure to aniseed flavor significantly affected food preference of the puppies after weaning at 10 weeks of age. They found prenatal flavor learning was lost by weaning. Puppies are typically not fed commercial puppy food until weaning age. As our assessments did not occur until 5 months of age, any early transient flavor preferences puppies may have had would have not been detected in this study and would not have provide useful information for puppy food development.

We plan to longitudinally follow our small dogs' preferences by repeating similar trials at mature age, senior, and geriatric life stages to monitor changes in puppy food preference across their lifespan. For longitudinal assessments we will use homemade kibbles to control for formulation changes over time that will likely occur for commercially produced products. The later in life that preferences for puppy foods persist, the more economical it would be for assessment centers to have adult dogs assess puppy foods for puppies. It is possible that with age, especially during the senior and geriatric life stage, that food preferences may change due to increased flavor learning or decreased olfactory and taste sensitivity. Hirai et al. (1996) reported atrophic changes with degeneration in the olfactory epithelium of dogs older than 14 years, and prominent changes in dogs over 17 years old. With age there was a decrease in the number of olfactory cells, of cilia of olfactory cells, and microvilli of supporting cells, which are likely to change dog odor sensitivity to foods. This notion in supported by Pelchat's (2000) finding that elderly dogs with poor olfaction showed less reluctance to try unpleasantly smelling foods.

Naturally, adult dogs cannot replace every aspect of food assessment for puppies. Notably, special diets formulated for medical conditions in puppies are important to be assessed by puppies. Likewise, it is not recommended that adult dogs are fed a regular diet of puppy food, particularly adult dogs that are overweight.

Conclusions

Taste preferences in small breed puppies at 5-8 months old were maintained when assessed again as young adults at 14-18 months old. This suggests that pet food developers do not need to have constant access to puppy panels to evaluate palatability of puppy foods. Rather, adult dog panels could be a quicker, more practical, and more economical option to aid pet food developers in getting a nutritionally balanced and tasty product to market that puppies would likely enjoy.

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Conflict of interest

This work was conducted by researchers employed by AFB International. AFB International produces pet food palatants. To prevent conflict of interest, all puppy foods offered in this study were commercially available and purchased from Pet Stores.

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Le preferenze alimentari del cucciolo sono mantenute nell'età adulta

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Sintesi

Le diete bilanciate che soddisfano i requisiti nutrizionali per le varie fasi della vita degli animali sono importanti per sostenere la salute poiché le esigenze alimentari cambiano con lo sviluppo. I proprietari di cani vogliono offrire ai loro cani una dieta gustosa ed equilibrata dal punto di vista nutrizionale. Gli sviluppatori di prodotti per alimenti per cani si sforzano di formulare tali diete in modo tempestivo e che soddisfino i requisiti nutrizionali specifici di un cane in varie fasi della vita. Le valutazioni della palatabilità durante lo sviluppo del prodotto possono essere costose e richiedere molto tempo quando si valutano alimenti appositamente progettati per più tipi di razza, dimensioni e età dei cani. La valutazione del cibo da parte dei cuccioli è particolarmente costosa perché esiste una finestra ristretta per le prove alimentari prima che raggiungano l'età adulta. Inoltre, non è pratico o ideale per i centri di valutazione dell'appetibilità acquisire continuamente cuccioli poiché restano tali solo per una breve frazione della loro vita. Pertanto, abbiamo valutato se le prove di preferenza su diete formulate specificamente per cuccioli di piccola razza potessero essere valutate da adulti di piccola razza e produrre risultati simili. Abbiamo eseguito sette prove di preferenza accoppiate per 14 giorni con venti cani di età compresa tra 5-8 mesi e di nuovo a 14-17 mesi. In sei delle sette prove, i cani erano coerenti nelle loro preferenze come adulti e come cuccioli. Sebbene non sia consigliabile che i proprietari di cani nutrano regolarmente i propri cani adulti con alimenti per cuccioli, i risultati suggeriscono che gli sviluppatori di alimenti per animali domestici non devono avere un accesso costante ai gruppi di cuccioli per valutare l'appetibilità degli alimenti a loro destinati. Piuttosto, i cani adulti potrebbero essere un'opzione più rapida, più pratica ed economica per aiutare gli sviluppatori di alimenti per animali domestici a ottenere sul mercato un prodotto che i cuccioli probabilmente apprezzerebbero.

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Hunting potential of the pointing dogs based on the junior hunting tests in Poland

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Abstract: Junior hunting tests evaluate the innate predispositions of the young dogs for hunting. Junior pointing dogs (<2 years old) are evaluated in six competitions: scent, pointing, searching, speed, swimming and obedience. The aim of this study was to analyse the influence of selected factors on performance of the young individuals. Analysis was based on the results of the junior hunting tests of the pointing dog collected over years 2007-2015 by the Polish Hunting Association. The database included 2107 individuals belonging to 26 breeds, such as German shorthaired pointer, Weimaraner, Irish setter, German wirehaired pointer, and Gordon setter. A nonparametric analysis of variance was performed to determine impact of age, sex and breed on dogs' performance. Spearman's correlation was carried out to estimate associations between traits. Breed influenced ($P < 0.01$) the total score as well as individual categories. The best performing pointing dogs were German shorthaired (87.74 points) and wirehaired pointer (86.78 points). The poorest results were achieved by English (64.23 points) and Irish setters (69.82 points). Sex affected the total score, speed ($P<0.05$) and swimming ($P<0.01$); the dogs performed better than bitches. Age did not have a significant impact on the results ($P>0.05$) except from swimming ($P <0.01$); older dogs produced higher scores. The most correlated categories were scent and pointing (0.754) as well as searching and speed (0.750). The data characterize the current status of the hunting potential of different breeds of pointing dogs in Poland and can be used as a tool in further breeding and training programs.

Key Words: gun dog, working dog, pointer, setter, hunting association.

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Introduction

Pointing dogs are specifically used for pointing and retrieving game during hunting. Their particular tasks include detection of birds, capture of birds, detection of scat, capture of mammals and detection of reptiles (Dahlgren et al., 2012). Based on official classification by International Cynology Federation (FCI), the group VII of pointing dogs contains 37 breeds. Different breeds of pointing dogs are divided into the British Isles (Pointer, Irish setter, English setter and Gordon setter) and the continental pointing dogs (German shorthaired pointer and wirehaired, Weimaraner, Hungarian vizsla, Small münsterländer, Brittany spaniel and Cesky fousek) (Monkiewicz & Wajdzik, 2003). The British Isles pointing dogs use the air scent, and the continental dogs use the ground scent. A characteristic feature of all the pointing dogs is assuming and holding a classical pointing position to show the location of the game to the hunter (Ciemniewski, 2013). The genetic improvement of the pointing dogs has resulted in extending their abilities and environments where they can effectively hunt. Contemporary pointing dogs can hunt not only in the fields, but also in the water (multilateral hunting/pointing dogs) and in the forest (versatile hunting/pointing dogs) (Brabletz, 2015).

Hunting in Poland has a long tradition and is a popular activity, due to extensive forest areas

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inhabited by different wildlife species (Gawin et al., 2015). The wild game population is managed by the Polish Hunting Association (PHA), which currently associates over 120 thousands of registered hunters. Hunting is allowed within over 4'698 hunting districts covering the area of 25'700.8k ha across the country. Many of those hunting areas are inhabited by feathered game, including pheasants (in 2016, 128k heads were shot) and partridges (in 2016, 2.7k heads were shot), which are hunted for using different breeds of hunting dogs. (Central Statistical Office, 2016)

Hunting potential and effects of training of the hunting dogs is also supervised by the PHA. To provide evaluation of the hunting dogs, PHA regularly organizes junior and senior hunting tests. Dogs are assigned to the hunting test based on their type and age. Junior hunting tests for pointing dogs is organised for young individuals of different breeds, from 9 to 24 months of age. Evaluating young dogs, prior to any hunting training, allows for determining their full hunting potential. The assessment of the hunting potential of the pointing dogs includes reaction to the shot, ability to catch and follow the scent, independence in hunting, pointing ability, obedience and reaction to the water. After passing the junior hunting test the dog can start the hunting training and obtain full breeding rights (PHA).

The literature on the pointing dogs is scarce and mainly related to their genetics or behaviour. Parra et al. (2008) compared five breeds of the Spanish pointing dogs and demonstrated high between-breed genetic diversity. Such high genetic diversity was most likely due to the genetically distinct individuals who shared a common ancestor, i.e., English setter with English pointer and German shorthaired pointer. Homozygosity mapping between pointing and herding dogs revealed that the pointing behaviour is most likely encoded by two polymorphisms on chromosome 22 (Akkad et al., 2015). Karlskov-Mortensen et al. (2019) identified a mutation responsible for blindness in Old Danish pointing dog, which leads us closer to understanding of the genetics of the pointing dogs. Behavioural research indicate that the pointing dogs belong to the least aggressive breeds (Stafford, 1996). Serpell & Duffy (2014) demonstrated that the German shorthaired pointer was not only the least aggressive breed, but also the least fearful. Pointing dogs, which are classified as sporting dogs, were evaluated as highly trainable, especially in comparison to non-sporting dogs (Turcsán et al., 2011). Arvelius & Klemetsdal (2013) developed a statistical model that allows for enhancing the potential for training English setter by breeding towards utility in cooperative hunting.

In this study, we aim to evaluate performance of the young pointing dogs in junior hunting tests, which are carried out for individuals prior to their training in hunting. Due to the increasingly frequent use of hunting dogs as companion dogs at work, we hypothesized that the hunting instinct in the pointing dogs could be disturbed. We also aim to determine, which breeds of pointing dogs express the highest hunting potential and whether we can pinpoint the demographic tendencies in the evaluated traits.

Materials and methods

Junior hunting tests and data collection

The dataset included results of the junior hunting test organized in Poland in 2007-2015 for the total number of 2107 pointing dogs. The major goal of the junior hunting tests is to evaluate hunting potential of the pointing dogs. For this reason, the animals must be young (9-24 months old) and without any prior hunting training. The data were derived from the "Catalogue of Junior and Senior Hunting tests", which is issued by the Polish Hunting Association (PHA). The individuals included in the junior hunting tests for pointing dogs must have been registered in the Polish Kennel Club. All the registered young dogs in the Polish Kennel Club must come from parents which have pedigrees and have birth certificate. The competitions were evaluated

by a committee consisting of judges registered in the Polish Kennel Club.

A junior hunting test for pointing dogs consists of six competitions: scent, pointing, searching, speed, swimming and obedience. Description of the junior hunting test and scoring criteria was included in Table 1. Junior hunting test begins with the dog's reaction to the gunshot (anxious dogs are excluded). The scoring scale ranges from 0 (the lowest note) to 4 (the highest note). Each score is multiplied by the weight corresponding to the difficulty of the given competition. The final result includes the sum of scores from the individual competitions. The dataset includes the following characteristics for each dog: name and surname of breeding dog, breed, sex, birth date, PKR number (number from Polish Pedigree Book) and name and surname of the owner. Weather and environmental conditions are not included in the scoring algorithm. Upon completing all competitions included in the hunting test, the dogs are ranked the certificates are issued.

Table 1. List of competition for the junior hunting test of the pointing dog

Trait	Score	Weight	Description
Scent	0-4	5	The dog should catch and follow the scent of the game
Pointing	0-4	5	The dog should freeze in a pointing position characteristic to a given breed
Searching	0-4	4	The dog should move in wide and zigzag pattern to follow the scent of the game
Speed	0-4	4	The dog should move characteristically for the given breed and as quickly as possible
Swimming	0-4	4	The dog should enter the water and swim about 5 m in 10 minutes (it is allowed to throw pebbles into the water to encourage the dog to enter the water)
Obedience	0-4	3	The dog should be willing to follow the instructions and keep in touch with the guide

The dataset was organized as follows: each dog was included in one age group only once. For dogs repeatedly involved in the junior hunting test, average results for the given year were used in statistical analyses. The animals were divided into four age groups: I (9-12 months old, 220 dogs), II (13-17 months old, 355 dogs), III (18-22 months old, 244 dogs) and IV (23-24 months old, 1288 dogs). The breed factor was analysed in case of groups > 50 individuals. The dataset contained categorical data, and therefore non-parametric statistical tests were used. The impact of sex, age and breed was analysed with non-parametric analysis of variations (NPANOVA). Differences between the groups were determined with the Mann-Whitney U test (for sex) or Kruskal-Wallis test (for age and breed). The correlation between competitions was estimated on the basis of Spearman's correlation coefficient. The calculations were made in the SAS statistical package (SAS Institute Inc, Cary, NC, USA) and Medcalc (MedCalc Software, Ostend, Belgium).

Results and discussion

Dog breeds

Analysis of the junior hunting test data was carried out for 2107 dogs belonging to 26 breeds. Figure 1 shows the distribution of the breeds involved in the junior hunting test for the pointing dogs. The most numerous breeds were: German shorthaired pointer (464 individuals), Irish setter

(348 individuals), and Weimaraner (323 individuals). More than half of the breeds (15 out of 26), including Large munsterlander, Wirehaired pointing griffon, Perdigueiro português, Braque saint-germain, and Slovak rough haired, were underrepresented (< 50 individuals). Those breeds were excluded from analysis of variance in which breed was a fixed factor. Dataset that allowed analysis of a breed effect comprised of 2012 individuals from 11 breeds. The distribution of the number of dogs in the given breeds participating in the junior hunting test of pointing dogs reflects the population of dogs registered in the Kennel Club in Poland. The most popular breed of all analysed in Poland is the German shorthaired pointer, which in 2015 had a population of 450 individuals, and the least popular breed was Brittany spaniel, 51 individuals (The Polish Kennel Club, 2017).

Figure 1. Distribution of the breeds involved in the junior hunting test for the pointing dogs.

Descriptive statistics

Descriptive statistics and the analysis of variance are presented in Table 2. The maximum achievable total score was 100, whereas the average total score obtained in this study was 79.38. The category that scored the highest mean was obedience (3.72), which was close to maximum value of 4 points. The lowest score was obtained for pointing (2.82). The total score reflected partial scores obtained by a given dog in each competition. Based on the literature, many factors influence the dog's hunting abilities, including the origin, relationship between the dog and the keeper and a balanced diet. Heritability of the hunting characteristics is varied. For example, the inheritance of obedience is very low and amounted to 0.02 in the Norwegian elkhound (Liinamo et al., 1997) and 0.04 in Norwegian elkhound grey (Wetten & Aasmundstan, 2014). The inheritance of pointing amounted to 0.25 in the German shorthaired pointer and 0.13 in the German wirehaired pointer (Schmutz & Schmutz, 2015). Karpiński et al. (2009) showed the influence of the maintenance method on adaptive intelligence. Dogs kept at home achieved higher Corena test results than dogs kept in the pen. Differences indicate that the dog's intelligence is also influenced by the relationship with the keeper (Karpiński et al., 2009). The effect of diet on the condition and endurance of the English pointing dogs during the hunting season was found (Davenport et al., 2001).

Table 2. Descriptive statistics and variance analysis of the junior hunting test for pointing dogs.

Value	N	Total Score	Scent	Pointing	Searching	Speed	Swimming	Obedience
Mean	2107	79.17	3.05	2.81	3.29	3.33	3.08	3.72
Min	2107	0	0	0	0	0	0	0
Max	2107	100	4	4	4	4	4	4
p-value								
Sex	2107	*	ns	ns	ns	*	**	ns
Age group	2107	ns	ns	ns	ns	ns	**	ns
Breed	2012	**	**	**	**	**	**	**

Differences between age groups and breed were examined using the Kruskal-Wallis test and between the sexes U-Mann Whitney test,
*p<0.05
**p<0.01
ns = not significant

Effects of sex, age and breed on hunting potential of pointing dogs

Table 3 shows effects of sex, age and breed on scores of the junior hunting test. Sex influenced (P<0.05) the total score as well as speed and swimming. Age group was significant (P<0.01) only in swimming. Breed was a significant factor (P<0.01) in total score and all individual categories.

Effects of sex on the junior hunting test

Effects of sex in the total score of the junior hunting tests for pointing dogs indicated that dogs outperformed bitches (P<0.05). We suppose that those differences can be attributed to the greater strength and durability of the dogs compared to bitches, as indicated by Brabletz (2015). The effects of sex was most pronounced in swimming (P<0.01) and speed (P<0.05). Those competitions that require more physical strength, which might explain better performance of the male dogs. Serpell & Hsu (2005) did not observe overall differences between sexes in training abilities, except from West highland white terriers and Dachshunds, in which dogs were more trainable than bitches. Lindberg et al. (2004) also reported higher scores in dogs than in bitches of Flat-coated retrievers, especially in the dog's reaction to the shot, reaction to the throw and cooperation.

Effects of age on the junior hunting test

Age did not have significant impact on the analysed scores of the pointing dogs (P>0.05), except from swimming. Individuals from the second age group (13-17 months of age) scored higher (P<0.01) than from the fourth age group (23-24 months of age), but the numerical difference was not too large (3.12 in group II vs. 3.07 in group IV). Lack of statistically significant influence of age on adaptive intelligence was demonstrated by Karpiński et al. (2009). It could be attributed to large ranges of the defined age groups. Lindberg et al. (2004) found more pronounced age effect on the hunting behavior of the Flat-coated retriever dogs. They found a significant impact of age on interest in searching and water retrieving as well as in searching efficiency, delivery and grip. However, no clear explanation of those effects was presented.

Table 3. The impact of the sex, breed and age on different competitions within junior hunting test of the pointing dogs.

Sex	n	Total Score	Scent	Pointing	Searching	Speed	Swimming	Obedience
Dog	875	80.12 [a]	3.05	2.86	3.32	3.38 [a]	3.21 [A]	3.72
Bitch	1233	78.49 [b]	3.04	2.77	3.27	3.30 [b]	2.99 [B]	3.73
Age group	n	Total Score	Scent	Pointing	Searching	Speed	Swimming	Obedience
1. (9-12)	220	80.25	3.11	2.89	3.35	3.40	3.02	3.73
2. (13-17)	355	79.33	3.04	2.81	3.30	3.31	3.12 [A]	3.75
3. (18-22)	244	78.36	3.01	2.78	3.25	3.31	3.09	3.68
4. (23-24)	1288	77.73	3.03	2.62	3.18	3.29	3.07 [B]	3.76
Breed	n	Total Score	Scent	Pointing	Searching	Speed	Swimming	Obedience
1. CF	58	$85.58^{5,7,11}$	$3.45^{5,7,8}$	$3.20^{5,7,11}$	3.45^{5}	3.34^{5}	$3.31^{5,7}$	$3.98^{2,5,7}$
2. GS	173	$79.00^{5,7,8,9}$	$3.00^{5,8}$	$2.68^{4,7,8,9}$	$3.36^{5,7,11}$	$3.39^{5,11}$	$3.15^{5,7,8,9}$	3.69^{1}
3. SM	80	$80.50^{5,7}$	$3.13^{5,7}$	$2.85^{5,7}$	$3.35^{5,11}$	3.33^{5}	$3.20^{5,7}$	3.72
4. P	88	$84.21^{5,7,11}$	$3.35^{5,7,11}$	$3.16^{2,5,7,11}$	$3.50^{5,7,11}$	$3.55^{5,7,11}$	$3.06^{5,7,8,9}$	3.76^{5}
5. ES	124	$64.23^{1,2,3,4,6,8,9,10,11}$	$2.40^{1,2,3,4,6,8,9,10}$	$2.17^{1,3,4,6,8,9,10}$	$2.72^{1,2,3,4,5,6,7,8,9,10}$	$2.83^{1,2,3,4,5,6,7,8,9,10}$	$2.27^{1,2,3,4,6,8,9,10,11}$	$3.41^{1,4,8,9,10,11}$
6. BS	54	$83.24^{5,7,11}$	$3.29^{5,7}$	$2.99^{5,7}$	$3.\ 56^{5,7,11}$	$3.66^{5,7,11}$	$2.98^{5,8,9}$	3.70
7. IS	348	$69.82^{1,2,3,4,6,8,9,10}$	$2.66^{1,3,4,6,8,9,10}$	$2.25^{1,2,3,4,6,8,8,10,11}$	$3.06^{2,4,5,6,8,9}$	$3.20^{4,5,6,8,9}$	$2.42^{1,2,3,4,8,9,10,11}$	$3.56^{1,8,9,10,11}$
8. GSP	464	$87.74^{2,5,7,10,11}$	$3.42^{2,5,7,11}$	$3.27^{2,5,7,11}$	$3.59^{5,7,10,11}$	$3.58^{5,7,11}$	$3.58^{2,4,5,6,7,11}$	$3.77^{5,7}$
9. GWP	160	$86.78^{2,5,7,11}$	$3.30^{5,7,11}$	$3.28^{2,5,7,11}$	$3.58^{5,7,11}$	$3.62^{5,7,11}$	$3.61^{2,4,5,6,7,11}$	$3.87^{5,7}$
10. HV	141	$82.21^{5,7,8,11}$	$3.15^{5,7}$	$3.00^{5,7,11}$	$3.26^{5,8}$	3.31^{5}	$3.40^{5,7,11}$	$3.84^{5,7}$
11. W	323	$74.09^{1,4,5,6,8,9,10}$	$2.81^{1,4,8,9}$	$2.54^{1,4,7,8,9,10}$	$3.01^{2,3,4,6,8,9}$	$3.03^{2,4,6,8,9}$	$2.93^{5,7,8,9,10}$	$3.82^{5,7}$

Effects of breed on the junior hunting test

Breed had the most significant influence on the total score as well as the scores of all competitions carried out for junior pointing dogs ($P<0.01$). The highest total score was obtained by the German shorthaired (87.74 points) and wirehaired pointer (86.78 points). These breeds are very popular working dogs in Poland due to their excellent air scent, big hunting passion and balanced character (Brabletz, 2015). On the other hand, the total performance of the English (64.23 points) and Irish setters (69.82 points) was the poorest. These breeds are characterized by elegant appearance, and in most cases they are used in Poland as companion dogs (Brabletz, 2015).

Results of the Competitions

*Scent***.** The highest results were achieved by Cesky fousek (3.45) and German shorthaired pointer (3.42), which are both characterized with excellent scent (Brabletz, 2015). Cesky fousek, known also as Bohemian wirehaired pointing griffon, is a rare, heritage breed originating from Czech Republic (Kuhn et al., 1977). During World War I it was close to extinction, but in the 1930s it was reestablished with admixture of German pointers (Kristin Mehus-Roe, 2009). English and Irish setters performed the poorest in the scent competition compared to other breeds (2.40 and 2.66, respectively). We can explain the poor performance by the fact that breeds are now used mainly as companion dogs, which could have driven the breeding more towards pleasant character rather than typical hunting abilities (Brabletz, 2015).

*Pointing***.** German shorthaired and wirehaired pointers scored high in pointing (3.27 and 3.28, respectively), which suggests their superior predispositions towards hunting. Schmutz and Schmutz (1998) obtained the same results for this competition for German shorthaired pointer and German wirehaired pointers (3.28). The North American Versatile Hunting Dog Association (NAVHDA www.navhda.org) publishes database with updated scores of the hunting tests conducted in the USA. In 2016, the breeds that produced the top scores in pointing competition were Hungarian vizsla (3.84), German shorthaired pointer (3.69) and German wirehaired pointer (3.57), while the lowest score was reached by the Weimaraner (3.07). German shorthaired pointers have been recognized as a breed since 1892, when the breed standards were written and introduced into German Kennel Club Stud Book (Vickie Lamb, 2015). German wirehaired pointer evolved from German shorthaired pointer. Nowadays, they belong to the most popular breeds of versatile hunting dogs worldwide, suitable for different type of work (i.e., hunt, point and retrieve both on land and in water). Both breeds have superior nose and speed as well as beautiful posture and social character.

*Searching***.** The highest score in the searching competition were obtained by Brittany spaniel, Pointer and German shorthaired and wirehaired pointers (3.56, 3.50, 3.59 and 3.58, respectively). The top scores were produced by the individuals able to search independently, covering a wide area and not sticking too close to the guide. Lower results of Weimaraner (3.01) could have been caused by mistake of maintaining too close contact with the handler, which is characteristic of this breed. Brenøe et al. (2002) analysed genetic parameters for hunting performance of the German shorthaired and wirehaired pointers and Brittany spaniel in Norway. Seeking width was evaluated during the test and it was based on the distance from the handler while hunting. On the scale from 0 to 6, German shorthaired pointer scored 3.24, German wirehaired pointer scored 3.33 and the Brittany spaniel – 3.26. Even though the scores reported by Brenøe et al. (2002) amounted to 66-68% of the maximum score (in our study the average score was app. 90% of the maximum), the three breeds evaluated performed on similar level, like in this study.

*Speed***.** Breeds that obtained the top results in this competition were Brittany spaniel, German shorthaired and wirehaired pointers (3.66, 3.58 and 3.62, respectively). This could have been

caused by rapid movement characteristic of these breeds. Lower performance of Weimaraner (3.03) might have been due to the slower pace and dynamics of walking attributed to their large size and moving in close contact with the handler (Brabletz, 2015). Brenøe et al. (2002) also reported similar score in speed competition for the three breeds that performed best in this study (the score of 3.90-3.95 amounted to 65-66% of the maximum score for this competition).

*Swimming***.** German shorthaired and wirehaired pointers scored the top results in swimming (3.58 and 3.61, respectively). German pointers are multi-purpose and versatile dog and the most popular breed among the gun dogs. English and Irish setters were the breeds that achieved the lowest score (2.27 and 2.42, respectively) despite being resistant to changes in the water temperature, and therefore entering the cold water should not cause them such a problem as dogs with a short coat. The differences between the Hungarian vizsla (3.40) and the Weimaraner (2.93) despite the similar body structure and coat are probably due to the fact that the Hungarian vizsla likes both terrestrial and aquatic environments (Brabletz, 2015).

*Obedience***.** Results of obedience were the highest and the most evenly distributed in this study. The reason for it is that the junior hunting tests evaluate willingness to obedience and maintaining contact with the handler rather than complete obedience, which is evaluated after the dogs undergo training (Polish Hunting Association). The working dog is the only domesticated animal that has been initially selected for behavior (Brabletz, 2015). The average result (3.72) was over 85% of the maximum number of points. Liinamo et al. (1997) found that the Finnish hound had a similarly high score (about 81% of the maximum score). In the studies of Wetten and Aasmundstan (2014) on the Norwegian breed of Elkhound grey, the animals achieved significantly lower scores (about 50% of the maximum number of points). Such results may be due to the fact that it is a primary breed and is characterized by high independence. Obedience of young dogs is influenced by many factors, including personality of the dog, socialization and relationship with the handler (Brabletz 2015).

Correlation between Competitions

Table 4 presents the Spearman's correlation coefficient between competitions in the junior hunting test of pointing dogs. A strong correlation occurred between scent and pointing (0.754), as well as between searching and speed (0.750). A strong correlation between scent and pointing is obvious and caused by the fact that when the pointing dog smells the game during scent competition, it should point at it right away. A strong correlation between searching and speed results from the pattern of movement while searching game animals. German shorthaired and wirehaired pointers move widely and quickly in the field. The Weimaraner, due to his moderate temperament, moves very dynamically and narrowly close to the handler. We also found poor or lack of correlation between obedience and other traits (i.e., speed or swimming). Even though results of obedience were very balanced between the breeds, other traits were varied as discussed earlier.

Table 4. Correlation between hunting traits evaluated during junior hunt test of the pointing dog.

Trait	Scent	Pointing	Searching	Speed	Swimming	Obedience
Scent		0.754	0.538	0.478	0.287	0.240
Pointing	0.754		0.510	0.425	0.314	0.250
Searching	0.538	0.510		0.750	0.303	0.210
Speed	0.478	0.425	0.750		0.296	0.177
Swimming	0.287	0.314	0.303	0.296		0.186
Obedience	0.240	0.250	0.210	0.177	0.186	

Conclusions

In this study we evaluated results of the junior hunting tests for pointing breeds. We found that in the category of young, untrained gun dogs, the top results were produced by the older males that belonged to German shorthaired or wirehaired pointers. It suggests that those breeds are the most suitable to perform the tasks of a working, versatile dog. The data characterize the current status of the hunting potential of different breeds of pointing dogs in Poland and can be used as a tool in further breeding and training programs.

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Abilità venatoria potenziale di cani da ferma sulla base dei test per giovani cani da caccia in Polonia

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Sintesi

I test per giovani cani da caccia valuta l'innata predisposizione venatoria dei giovani cani. Giovani cani da ferma (<2 anni di età) sono valutati in 6 competizioni: fiuto, punta, ricerca, velocità, nuoto ed obbedienza.

Lo scopo della ricerca è stato quello di analizzare l'influenza di fattori selezionati sulle performance dei giovani animali. L'analisi è stata basata sui risultati dei test su giovani cani da ferma raccolti nel periodo 2007-2015 dall'Associazione Polacca della Caccia.

Il database includeva 2107 individui appartenenti a 26 razze. L'analisi non parametrica della varianza è stata effettuata per determinare l'impatto di età, sesso e razza sulla performance del cane. La correlazione di Spearman è stata effettuata per stimare le associazioni tra i tratti. La razza ha influenzato (P <0,01) il punteggio totale e le singole categorie. I cani da ferma con le migliori prestazioni erano i pointer tedeschi a pelo corto (87,74 punti) e a pelo ruvido (86,78 punti). I risultati più scarsi sono stati raggiunti dai setter inglesi (64,23 punti) e irlandesi (69,82 punti). Il sesso ha influenzato il punteggio totale, la velocità (P <0,05) e il nuoto (P <0,01); i maschi si sono comportati meglio delle femmine. L'età non ha avuto un impatto significativo sui risultati (P> 0,05) ad eccezione del nuoto (P <0,01); i cani più anziani hanno prodotto punteggi più alti. Le categorie più correlate erano fiuto e ferma (0,754), nonché ricerca e velocità (0,750). I dati caratterizzano lo stato attuale del potenziale di caccia di diverse razze di cani da ferma in Polonia e possono essere utilizzati come strumento in ulteriori programmi di allevamento e addestramento.

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Effect of lavender (*Lavandula angustifolia*) essential oils on sheltered dog behavior: preliminary results

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Abstract: Dogs in a shelter environment need to cope with different types of stressors. Recent studies have shown that essential oils might be able to modify the behavior of dogs. The aim of the current study was to assess whether olfactory enrichment through lavender essential oils influences the behavior of sheltered dogs. 11 dogs (7 males and 4 females, castrated), aged between 18 months and 13 years were involved. Animals were divided into two groups: G1 (Experimental group and G2 (Control group).
G1 dogs wore a collar with a gauze on which 5 drops of lavender (*Lavandula angustifolia*) essential oil were laid once a week. The dogs of G2 wore a collar identical to those of G1 but without aromatization with lavender. The dogs underwent a 10-minute isolation period (T0) in an unknown environment, then they were brought back to their own box and their behavior was videotaped for 5 minutes. The same procedure was repeated after one (T1) and two (T2) months. A significant difference among the data of stress behaviors recorded at the different times is observable in dogs G2 (χ^2= 6.00; p=0.05), while no difference was observed in experimental dog G1 (χ^2= 0.857; n. s.).
Preliminary results of this study seem to indicate that the application of a collar impregnated with lavender essence can have a positive effect on the stress of the animal in the kennel, avoiding an increase in stress behaviors, as happens instead in the control group.

Key Words: dog lavender, essential oil, shelter, stress

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Introduction

Domestic animals, especially the dogs, have shared their lives with humans for a long time (Gazzano, 2014). However, this relationship is sometimes negatively influenced by the exhibition of 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).

Dogs in a shelter environment need to cope with different types of stressors: social (reduced intraspecific and/or interspecific social contacts), environmental (restraint for medical procedures, separation from a caretaker or handler) or psychogenic stressors (separation anxiety, use of aversive training methods by a previous owner/lack of ethological knowledge in caretakers) (Cozzi et al., 2016).

Recent studies have shown that essential oils might be able to modify the behavior of dogs and cats (Wells, 2004; Graham et al., 2004; Wells & Egli, 2015; Binks et al., 2018). In these studies, the welfare measurements included physiological indicators, such as corticosteroid levels (Beerda et al., 1998) or behaviors related to chronic stress, such as repetitive behaviors, nosing, paw-lifting, increased locomotion, displacement behavior or excessive drinking (Beerda et al., 1998; Haverbeke et al., 2008).

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The aim of the current study was to assess whether olfactory enrichment through lavender essential oils influences the behavior of sheltered dogs.

Material and methods

In this research 11 dogs (7 males and 4 females, castrated), aged between 18 months and 13 years were involved. Animals were divided into two groups: G1 (Experimental group) consisting of 7 Animals (5 males, 2 females) and G2 (Control group), consisting of 4 Animals (2 males, 2 females).

G1 dogs wore a collar with a gauze (Fig. 1) on which 5 drops of lavender (*Lavandula angustifolia*) essential oil were laid once a week. The dogs of G2 wore a collar identical to those of G1 but without aromatization with lavender.

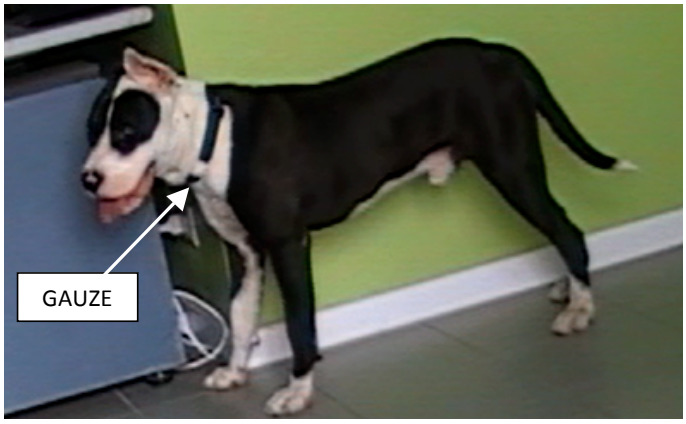

Figure 1. Dog wearning a lavander collar

The dogs underwent a 10-minute isolation period in an unknown environment, then they were brought back to their own box and their behavior was videotaped for 5 minutes (T0). The same procedure was repeated after one (T1) and two (T2) months.

For the analysis of dog behaviors, the ethogram previously described by Cozzi et al. (2016) was used, evaluating the total duration (seconds) of 13 behaviors considered realistic stress indicators (orientation at the door, turning the head, frequent and labored breathing, scratching the door, yelping, licking parts of the body, barking, elimination of feces and /or urine, tail between paws, scratching, shaking, licking the lips and howling). Statistical analysis was performed using Friedman and Wilcoxon tests.

Results

The figures 2 and 3 show the data relating to stress behaviors in the two groups considered at the different detection times.

A significant difference among the duration in seconds of stress behaviors recorded at the different times is observable in dogs G2 (χ^2= 6.00; p=0.05; fig. 2), while no difference was observed in experimental dog G1 (χ^2= 0.857; n. s.; fig. 3).

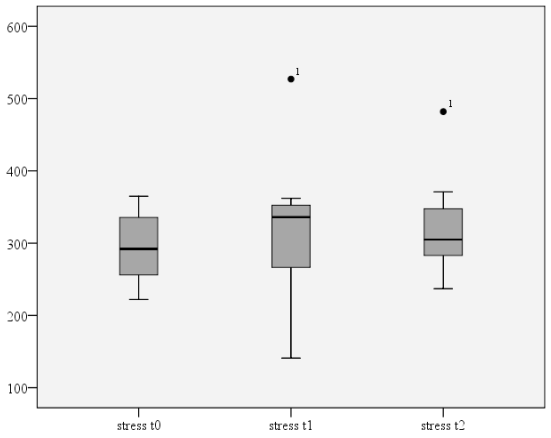

Figure 2. Duration (seconds) of the signs of stress displayed in the different times in a control group

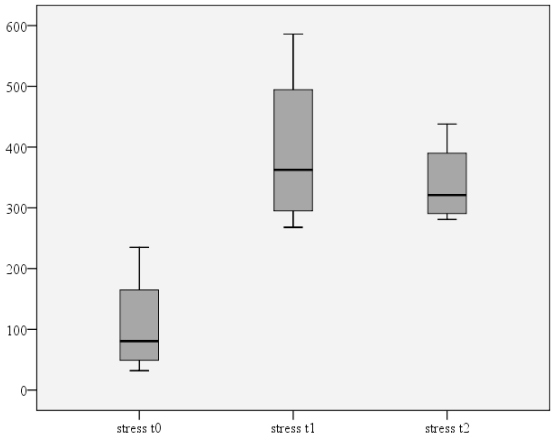

Figure 3. Duration (seconds) of the signs of stress displayed in the different times in a experimental group

Discussion

The dog has been sharing his life with man for many years and has undergone very important physical (Gazzano et al., 2013) and psychological changes. This animal is able to develop a strong attachment bond with the human being (Mariti et al., 2013), as well as with conspecifics (Mariti et al., 2018).

However, this relationship is sometimes interrupted, and the dog is relinquished in a shelter where he can stay for a long time. Rescue and rehoming shelters are frequently stressful and impoverished environments.

While diet manipulation seems to fail in improving dog behavior (Gazzano et al., 2019), other studies (Uccheddu et al., 2018) have shown the positive effect of the diffusion of essential oils on the affective states of the dog, but the effect of a collar impregnated with fragrant essences on the dog's behavior has never been evaluated.

Preliminary results of this study seem to indicate that the application of a collar impregnated with lavender essence can have a positive effect on the welfare of the animal in the kennel, avoiding an increase in stress behaviors, as happens instead in the control group.

Further studies will be needed to confirm these results, using a larger number of animals.

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Effetto dell'olio essenziale di lavanda (*Lavandula angustifolia*) sul comportamento del cane: risultati preliminari

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Sintesi

I cani in canile devono affrontare tipologie differenti di stress. Studi recenti hanno dimostrato che gli olii essenziali potrebbero essere in grado di modificare il comportamento del cane.

Lo scopo della presente ricerca è stato quello di verificare se un arricchimento olfattivo attraverso l'utilizzo di olio essenziale di lavanda, possa influenzare il comportamento di cani in canile.

Per la ricerca sono stati utilizzati 11 cani (7 maschi e 4 femmine, castrati) di età compresa tra i 18 mesi ed i 13 anni.

Gli animali erano divisi in 2 gruppi: G1 (gruppo sperimentale) e G2 (gruppo controllo). I cani del gruppo G1 indossavano un collare su cui era stata posizionata una garza che ogni settimana era impregnata con 5 gocce di olio essenziale di lavanda.

I cani del gruppo G2 indossavano un collare identico a quelli G1 ma non impregnato di olio essenziale di lavanda.

Gli animali erano sottoposti ad un periodo di 10 minuti di isolamento (T0) in un ambiente sconosciuto, quindi erano riportati nel proprio box ed il loro comportamento era videoregistrato per 5 minuti.

La stessa procedura era ripetuta dopo 1 (T1) e 2 (T2) mesi.

Una differenza significativa è stata rilevata tra la durata dei comportamenti di stress tra di versi tempi di osservazione nel gruppo G2 (χ^2= 6,00; p=0,05), mentre nessuna differenza è stata osservata nel gruppo sperimentale G1 (χ^2= 0,857; n. s.).

I risultati preliminari di questo studio sembrano indicare che l'applicazione di un collare impregnato con olio essenziale di lavanda può avere un effetto positivo sullo stress dell'animale in canile, evitando un incremento dei comportamenti di stress, come avviene invece nel gruppo di controllo.

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The psychological and emotional care of the orphaned puppy: the management and the risk of behavioural disorders

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Abstract: Under the loving and expert guidance of the mother, who knows how to educate its playful experiences, the puppy grows emotionally balanced, learns to be autonomous, to control its exuberance and to relate properly with conspecifics and not. Without the mother, a human being has to take care of the orphaned pup.

The caregiver has to ensure to the puppy not only its physical health, but also its psychological health, in order to grow psychologically well-balanced subjects. The people that decide to take care of orphaned puppies must be aware and should emulate, as far as possible, the maternal behaviour. The studies about sensitive period has allowed to identify periods of the puppy's growth during which a specific stimulus reaches its maximum effect. The socialisation period is the hardest one. The caregiver has to give to the pup the possibility of knowing both conspecifics and heterospecific and ensure to it an enriched style of life.

Despite all the good will, the caregiver's action has inevitable shortcomings and possible errors. However, it is essential that the human helps the orphan in its psychic maturation, in order to prevent pathological behaviours. Especially the caregiver should manage correctly the attachment and detachment, to avoid the development of fears, anxieties, phobias and those syndromes that are caused by an alteration of sensory homeostasis, such as sensory deprivation syndrome, hypersensitivity-hyperactivity syndrome, primary desocialization, stereotypes and compulsive disorders, depression and aggressions.

Key Words: Behavioral disorder, caregiver, orphaned pup.

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Introduction

The causes that make the puppy an orphan can be various. Most often, the death of the mother or the abandonment of the puppies are the principal cause. Sometimes the mother is alive, but she can be ill or can assume an altered behaviour towards her offspring, such as aggression or a refusal of the litter (Peterson, 2011; Veronesi, 2013). The first thing to do in front of orphan puppies is the assessment of their age. The age is a fundamental factor that the caregiver should know, because it is important to choose the care and attention to be given to puppies. In order to establish the age of puppies, such as in case of abandonment, there are some physiological parameters that can be use (table 1) (Casal, 2012; Veronesi, 2013).

The physical and behavioural development of the puppy is divided in different sensitive periods, which partly overlap. The start and the ending of each phase are biologically determined and can change according to the breed or to individual characteristics. The definition of these periods varies between the authors (table 2) (Lindsay, 2000; Battaglia, 2009).

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Table 1. Physiological events useful in the assessment of puppies' age (Veronesi, 2013).

Detachment of umbilical cord	< 3 days
Flexing hyper tonus	< 4 days
Extensor hyper tonus	4 days – 28 days
Eye opening	8 days – 16 days
Opening of auditory canals	6 days – 14 days
Eruption of deciduous teeth	14 days – 21 days
Reflexes of suction and portage and ano-genital reflex.	21 days – 28 days

Table 2. Sensitive periods define by the authors.

Prenatal Period	from the 45th day of gestation to the birth		Champagne, 2008; Heim and Binder, 2012; Serpell, 2017.
Neonatal Period	from the birth to 12 days	Scott and Fuller, 1965; Scott et al., 1974.	Champagne, 2008; Heim and Binder, 2012; Serpell, 2017.
Transition Period	from13th to 21st day	Scott and Fuller, 1965; Scott et al., 1974.	Champagne, 2008; Heim and Binder, 2012; Serpell, 2017.
Socialization Period	from 22nd to 84th day	Scott and Fuller, 1965; Scott et al., 1974.	Champagne, 2008; Heim and Binder, 2012; Serpell, 2017.
Juvenile Period	from 84th day to sexual maturation	Scott and Fuller, 1965; Scott et al., 1974.	Champagne, 2008; Heim and Binder, 2012; Serpell, 2017.
Pubertal Period	from puberty to the 14th month		Champagne, 2008; Heim and Binder, 2012; Serpell, 2017.

During these periods, learning process and the establishment of stable and lasting association are facilitated (Scott & Fuller, 1965). The theory of sensitive periods underlines that dog behaviour evolve during the life of the individual, according to its experience and the environmental stimuli. While the puppy grows up, the experiences that it lives during the sensitive periods influence and determine its behavioural phenotype (Case, 2010; Battaglia, 2009). An adult dog that during the sensitive period has not received the correct stimulation, can show difficulties to modulate its sensorial responses, responding in the same manner to stimuli of different intensity (Pageat, 1999).

The theory of sensitive periods finds its deep explanation into the ontogenesis of the central and peripheral nervous system, whose development is determined by the genotype, but it is modelled by external stimuli. The development of nervous system can be divided into two period:

The first phase, when neurons are formed, proliferate, differ themselves and migrate in their final location. This process occurs mostly during embryonal and foetal life. However, some brain's area continues to develop even after birth, such as the dog's occipital cortex, which at birth is still composed by numerous immature neuroblast and reaches the complete development at 3 weeks of age (Fox, 1965). Also, neurons from the olfactory region and of the hippocampus continue to mature in adulthood (Whitman & Greer, 2009);

The second phase is represented by the formation of the connections of axons and dendrites. This process, that is named synaptogenesis, is extremely important, because neural connections begin to be formed already in pre-natal life, but they continue also after the birth. This connection

between neurons are redundant, so they develop in excess. (Pageat, 1999). It has been assumed that this redundancy of synapsis represents a biological substrate already prepared to receive the transcriptions of a lot of stimuli or that it may be a sort of backup in case of damage (Huttenlocher, 1984). After this phase of redundancy, there is a phase of selective stabilisation of synapsis. This period is delicate: the unnecessary connections that do not received any external stimulus are delated, and the connection activated by the external stimuli survived. The external stimuli have a retroactive effect of reinforcement on the synapsis (Pageat, 1999).

During its ontogenesis, the brain increases its volume not for the number of new nervous cells, but mostly for the connections that born towards them. A puppy grown in a hypostimulant environment will have fewer connections and its brain's size will be littler (Coppinger & Coppinger, 2001). Therefore, the theory of sensitive periods explains why there are differences in the abilities and performance of individuals of the same species (Battaglia, 2009).

The management of orphaned puppies

Finding a surrogate mother of the same species is the best solution for orphaned puppies care. Fostering can ensure normal maternal care and the correct behavioural development of the puppies. However, it is not so simple to find another bitch that easily accepts the puppies (Casal, 2012; Veronesi, 2013; Peterson, 2011). Therefore, most of the times is a human being that must replace the mother, and one of the most demanding part in the care of puppies is to ensure to them a normal emotional development. This aim is difficult because the caregiver should manage the phases of attachment and detachment, two fundamental steps of psychological development of the dog (Giussani, 2005; Casal, 2012). For the orphaned puppy the companionship of its brothers or sisters is a great advantage. Indeed, in the first days of life other puppies are a source of heat and tactile stimuli. In the following weeks they are playmates and share new experiences. This helps a proper behavioural development and the learning of the fundamental rules of their existence (Gazzano, 2008; Luescher, 2011).

Therefore, especially when orphaned puppies remained alone, it is fundamental that the caregiver learns how to ensure a correct physical and psychological growth (Giussani, 2005). For example, Marchesini (2007) underlines how the respect of sleep-wake rhythm is important for the correct neurologic development and the pup's cognitive potentiality.

Both the lack of the exposure to appropriate stimuli and the experience lived in the wrong moment can alter the behaviours of puppies (Overall, 2013). If the experience is not lived in the right moment, it will define dyschronia, whereas the experience not lived is called privation. Finally, the experience removed before the time is defined deprivation (Marchesini, 2007).

The difficulties of the caregiver

During the prenatal period, the human intervention is limited, and it is focussed on ensuring the mother wellbeing. During the first two weeks, the neonates have a tight bond with their mother, that the caregiver cannot fully replace. A premature or prolonged separation from the mother can cause the transitional or permanence development of obsessive-compulsive behaviours and stereotypies, due also to neural modification in the *substantia reticolaris* and changes in the neuroendocrine response (Lindsay, 2000; Giussani, 2016).

In the first days of life, the licking of perineal area by the bitch has the goal to help the puppy to defecate and urinate. After the third week, this behaviour represents an experience for the learning and memorization of the submission posture, fundamental into the communication between intraspecific. If the caregiver cannot imitate this maternal behaviour, there will be the risk that the puppy becomes unable to communicate with the conspecifics, showing aggressiveness or anxious (Giussani, 2016).

The mother educates the puppies to the self-control and helps them to establish different points of reaction to face different stimuli, so they acquire a state of emotional equilibrium. The caregiver should replace the mother, but often he doesn't have her educational coherence. The human should provide the puppies gradual stimuli permeated of positive connotations and teach them the self-control. If the environment is poor of stimuli, it can be the origin of the sensory deprivation syndrome and the puppy can develop hypersensitivity, excess of reactivity, alert, fear and avoidance response. The lack of self-control can lead to the hypersensitivity-hyperactivity syndrome, and the subject will show an excessive emotional arousal, for example in response to tactile stimulations (Marchesini, 2007; Giussani, 2016).

The primary socialization gives to the puppy an identity of species and it learns how to communicate adequately with conspecifics (Dehasse, 1994). In future, the orphaned puppies that have not the possibilities of interaction with other dog during this period can show nervousness, fear and aggressive behaviours in presence of conspecifics (Seksel, 1997; Serpell, 2017). From the seventh week the pup imitates the mother and the brothers, learning most quickly new behavioural modules towards the activation of the mirror neurons (Antoni & Tarricone, 2002). The caregiver lack of the isomorphism, which is the base of the imitation learning, so the puppies can be supported by a well-balanced dog. If this does not happen and the pup has not any contact with other dogs, it can identify itself in another species, such as human, and develop avoidance behaviour towards conspecifics (Dehasse, 1994).

Without the mother, the attachment happens toward the caregiver (Peterson, 2011). The person who takes care of the pup should teach it the detachment, in order to avoid the development of an excessive attachment, insecurity and morbid behaviours. The pup must acquire independence and the capacity of get over the daily challenges. The caregiver, for example, can avoid the puppy to lies down in contact with him/her, or can decide to ignore the pup or leave it alone for period of time gradually longer, in order to teach him to manage the feeling of abandonment (Dehasse, 1994; Giussani, 2005; Luescher, 2011).

During the secondary socialization the pup learn to recognize human as social partner, to distinguish the difference between men, women, kids and old people, and to instore a correct interactive style. In the meantime, it gets familiar with the environment where it lives. In this cataloguing phase, the young dog must be exposed to the greatest possible number of events, so that, when it will be adult, it will be able to recognize and catalogue every element, with which set up a correct relation. If its knowledge repertoire is poor and inadequate, the adult dog risks to show pathological behaviours (Serpell, 2017). From the 8th week the fear begins to arise, the subject that is not trained to relate with different stimuli can undergo a strong sensibilization, with considerable emotional fluctuations unsuitable with the intensity of the new stimuli (Lindsay, 2000; Luescher, 2011). Also after its adoption, the caregiver or the future owner should to ensure to the puppy an adequate environmental enrichment and the safe exposure to different experiences, in order to stimulate a normal neurologic and emotional development, to ensure a certain degree of mental stimulation and to grow up an emotional stable individual (Gazzano et al., 2008; Luescher, 2011). For example, to facilitate the acquisition of a correct intra- and interspecific communication, they have been conceived the puppy classes (Seksel, 1997; González-Martínez et al., 2019). Also, the teaching of sample orders, such as "rest" or "sit down", can help the owner and the puppy to have a better control of the situation. An inadequate quality and quantity of stimulation of the puppy can lead to the development of fear and anxious behaviours, with aberrant response to stressful situation (Lopate & Seksel, 2012). The individual learns to distinguish relevant or not biological and social stimuli through the exploration of different stimuli. Indeed, the subject hand-raised exposed to a variation of environmental stimuli (unknown persons, other animals and sensorial news) results more prepared to the exploration of new situation with more serenity compared to the dogs raised in a lab (Wright, 1983).

After the adoption, the owner has to assume the educational task, to prevent a regression of

the puppy and the onset of exuberant and undesired behaviours. If the education intervention of the owner is inadequate or lacks, the dog will assume wrong behaviours, without the respect of social rules and with emotional fluctuation. Anxious behaviours, sample or complex phobia, self-control reduction or lack of detachment can occur (Marchesini, 2007).

Behavioural disorders caused by an inadequate psychological and emotional care of the orphaned puppy

Aggressiveness

A unique definition of aggressiveness is difficult to identify (Reisner, 2004). According to Case (2010), aggressiveness can be defined as an agonistic behaviour characterized by a high arousal, that a subject uses to hurt another subject, which can react with aggressiveness or flying away. The aggressiveness is a normal behaviour of the dog, which uses it to communicate its emotional discomfort, using postures and warning signs (Capra & Robotti, 2007). The ritualization of the behaviour has led to the development of warning signals that, if correctly understood by the recipient, will prevent the real aggression (Horwitz & Mills, 2012, pp 13-16; Mège et al., 2003). The normal aggressive sequence has four phases: appetitive phase (threat, s.e. the growl), consummatory phase (s.e. bite), phase of cessation and rebalancing and the refractory period (Mège et al., 2003).

The dog that shows inappropriate aggressive behaviours out of context has to be considered clinically abnormal (Overall, 2013). An aggression can be defined pathological when it is an abnormal response compared to the stimulus, when the threat phase is absent or really brief, when the signs of pacification of the opponent are ignored and when the dog chooses to act the aggression, although it can have other alternatives (Ruffoni, 2018).

The canine aggressiveness can be determined by a genetic component, by neurologic or painful pathologies, or by negative experiences lived during the first weeks of life (missed learning of self-control and alimentary hierarchy, environment poor of stimuli, or insufficient intra-interspecific socialization) (Violet, 2016). Moreover, restlessness, anxiety, fear, great stressful situations, frustration, irritability and anger can lead to the manifestation of aggressive behaviours, when the dog is not able to slip its discomfort (Lindsay, 2001, pp. 166-169).

Reisner et al. (1996) and Cakiroglu et al. (2007) have demonstrated the correlation between aggressiveness and low level of serotonin in the cerebrospinal liquid and Rosado et al. (2010) in plasma. Even though an increase in TRP/5LNAAs ratio in plasma could influence the 5-HT levels in the brain and, consequently, the diet could have a positive effect on some dog behavioural problems (Gazzano et al., 2018), the use of medications like fluoxetine, a Selective Serotonin Reuptake Inhibitor (SSRI), is one of the commonly remedy in small animal behavioural medicine (Kaur et al., 2016).

Fear, Phobias and Anxiety

The fear is a strong emotional reaction towards a specific, real and well-identified danger. This is a normal response useful to the survival of the subject. If the reaction is excessive and without reason, the fear loses its adaptive value and became a pathological condition, identified as phobia (Case, 2010; Landsberg et al., 2013).

A phobic subject can't have a normal lifestyle. Stimuli that have a vital importance for the species and derived from unborn mechanisms can cause exaggerated fear reaction, because of a sensibilization process and anticipation mechanism (Beaver, 2009). The French School distinguishes post-traumatic and ontogenic phobias. The first arise for the exposition of the subject to an intensive stimulus in a closed environment, the second are due to the experience that the animal has not done during the development phase (Colangeli & Giussani, 2004).

The phobias can be simple, complex or social (Beaver, 2009). The simple phobia is toward to well-identified event, whereas in the complex phobia the stimulus that causes fear responses is difficult to identified, because of emotional anticipation process. This can evolve in spontaneous recovery, instrumentalization or anxiety (Colangeli & Giussani, 2004; Beaver, 2009; Ruffoni, 2018). The social phobia can be simple or generalized and can be towards conspecifics or heterospecifics, such as towards people (Beaver, 2009).

Anxiety is a pathological state more incapacitating than phobia. Although it is characterized by reaction similar to the fear, it is not cause by really dangerous stimuli. It is an apprehensive state with emotional anticipation and fear reactions towards a possible future danger (Case, 2010; Landsberg et al., 2013). In the anxious state there is a disorganization of the self-controls and a loss of adaptation to any changes in the external or internal environment (Colangeli & Giussani, 2004).

Fear, phobias and anxiety compromise the well-being of the dog. The subject is not able to adapt to minimal variation of the environment, it lives in a continuous state of alert and can develop syndromes with different signs, but all originated by an alteration of sensorial homeostasis.

Sensory deprivation syndrome

The sensory deprivation syndrome is characterized by an incapacity of managing the sensory information that comes from the environment. A missed maternal educational support or an inadequate variety of stimuli offered by the caregiver during the socialization period exposed the puppy to develop this pathology and to have an exaggerated fear reaction in front of new stimuli. The dog has not a rich database of experience and it is not able to make the correct cataloguing of the stimuli, remaining in a continuous state of insecurity (Mège et al., 2003; Beata, 2006; Luescher, 2011).

Table 3. Stage of the sensory deprivation syndrome.

	Stage 1 **Phobic stage or Stage of ontogenic phobia**	**Stage 2** **Anxious stage or stage of permanent anxiety**	**Stage 3** **Depressive stage or chronic depressive stage**
The stimuli that trigger the fear reaction or the anxious state	Well-identified stimuli such as humans, dogs, objects' noises.	Different stimuli that are difficult to recognize. It is the evolution of the phobic stage.	It is a rare condition
Symptoms and dog's reactions	Hypervigilance, avoidance, escape and /or defensive aggression. Activation of adrenergic system (dilated pupils, tremors, polypnea, tachycardia).	Inhibition, avoidance and escape and aggression for fear. The explorative behaviour is restricted, and the dog shows the posture of the expectation. The subject is unable to adapt to changes (environments, people and objects) and it doesn't walk away from its secure place. The dog can defecate and urinate at home, and eats during the night, when not observed.	General culling, resulting in the disappearance of exploratory and play activities. There are also alterations of sleep, involuntary eliminations in inappropriate places
Evolution	It is a dynamic stage and the dog can manage the stimulus and heal spontaneously. It can switch from a simple phobia to a complex phobia. The repeated exposure to the triggers strengthens and worsens phobic state	Often replacement activities can emerge, and they can lead to self-injuries. As adaptive mechanism, the hyper attachment to the owner can arise, and the absence of the owner can cause panic attacks.	

This syndrome has three different clinical presentation, corresponding to three different stages of emotional degradation described in table 3 (Mège et al., 2003; Beata, 2006; Beaver, 2009; Giussani, 2016).

The therapy is long and often there are relapse. It differs according to the animal's resources and the condition of the family and environment. They can be used social and environmental enrichment, cognitive-relational activities. It can be employed a pharmacological therapy (idrolized alfa-S1-Kasein, selegiline, clomipramine, fluoxetine) and/or a pheromonal therapy. What for the family nucleus can be an insignificant change, for the dog can represent a great goal (Giussani, 2016).

Hypersensitivity-hyperactivity syndrome

The Hypersensitivity-Hyperactivity syndrome (HS-HA) is a pathological condition depending on the disfunction of the sensory filter, which causes a deficit in the emotional management and an alteration of the motor control. It follows a typical sequence: hypersensitivity, hyper-emotionality, hyperactivity. This pathology is caused by the growth of the dog in a hypo-stimulant environment. In this condition, the sensory filter, which is in the *substantia reticularis*, has not the possibilities of development. The pup is not able to give different importance to different stimuli, so it reacts to everything in the same manner (Pageat, 1999; Giussani, 2016). The hyperactivity derived from the missed learning of self-controls, so the dog cannot activate the stopping sequences and the consummatory phase lengthens (Ozella & Pessani, 2015).

The pup learns the self-control from the mother when it is 2-3 months old. The mother stops the action of the pup when its arousal is too high and can became self-defeating. She teaches to her puppies to handle waiting and frustration. In case of orphaned puppies, this teaching lacks and the puppy has a chaotic communication, because it wants to communicate a lot of things at the same time, all with the same importance. Failing to do it adequately, the pup can become aggressive (Giussani, 2016; Lindsay, 2001).

The alteration of the taste can cause hyperphagia (Ozella & Pessani, 2015). However, if it is associated with phobia, anxiety or sensory deprivation syndrome, there will be dysphagia and anorexia (Colangeli & Giussani, 2004). Regarding the sight, the pup can develop its predatory instinct and wants to follow everything, pulls the leash and reacts with anger or fear towards big noise object that moves. The dog is in a state of continuous hypervigilance and can't sleep. A tactile stimulus (simple touch or a friendly pat) can cause an excessive reaction and the pup can bite. The deficit of the filter on the olfaction causes an inadequate comprehension of the scent. Finally, a familiar sound bounded with positive activities, can cause confusion, excitement and the increase of the arousal (Giussani, 2016).

The continuous state of excitement and hypervigilance leads the dog to show uncoordinated, excessive, violent and invasive behaviours (Lindsay, 2001).

The hyperactive pups haven't got problems of comprehension, but they have difficulties with memorization and elaboration. The difficulties increase according to the arousal of the subject, and it is easier to memorize negative emotions, joint with fear and suffering. Therefore, it is important to work with a correct behavioural and cognitive-relational therapy, based on the use of positive reinforcement. The behavioural therapy can be supported through a pharmacological therapy, to reduce the anxiety and improve the concentration and the learning, such as the use of serotonin reuptake inhibitors, such as the clomipramine or fluoxetine. Reducing the arousal fluctuation and the impulsivity allows to the dog to think before to act (Pageat, 1999; Mège et al., 2003; Masson & Gaultier, 2018).

Separation-related disorders and separation anxiety

The separation-related disorders derived from an emotional incompetence in the management of loneliness. According to the severity of the disorder, it arises when the owner is absent or when there is an obstacle between the dog and the owner, such as a close door (Blackwell, 2016). The anxiety that the puppy shows when it walks away too far from its mother has got a biological adaptive meaning. It is possible that the domestication and the genetic selection have led to very social subjects, which depend a lot from human beings (Horwitz, 2009). Moreover, this dependence can be strengthened by the requests by humans that anthropomorphise the dogs and ask them to assume childish roles (Serpell, 2017).

The etiological factor that cause the onset of the pathology is recognized by a sudden separation of the dog from the owner, preceded by an exclusive and constant human-dog contact. What is the cause of the development of separation-related disorders probably can be explained with defects in the separation process from the mother or form the figure of attachment, during the first months of dog's life (Serpell, 2017; Pierantoni et al., 2011). Pageat (1999) and the French school argue that the cause can be found in an excessive quantity of attachment (hyper attachment) between man and dog. The bond between the owner and its dog can be like the relation between a mother and her offspring, because the owner or the caregiver become a reference figure for the puppy (Topàl et al., 1998). Flannigan & Dodman (2001) have demonstrated that dogs who live with a single owner can show more often behaviours that characterized separation-related disorders compered to conspecifics that live in more complex familiar nucleus. Finally, Parthasarathy & Crowell-Davis (2006) assume that the separation anxiety is not caused by an excessive of attachment, but by an inappropriate relation dog-owner.

The dog that suffers this pathology shows an excessive request of visual and physical contact with its owner, looking for continuous reassurance from the family (Beaver, 2009). The symptoms are analogous to the manifestations of a panic attack, with continuous attempts to re-join with the figure of attachment (Ogata, 2016). When the dog recognizes the preparation of an imminent exit of the owner, it begins to follow the human in all the rooms, it is worried and pants, and it omits to eat. When the owner leaves the dog alone, it manifests its discomfort with a complex and various repertoire of excessive, repetitive and prolonged vocalization (barking, whining). They can be followed by the attempt to reach the owner, and, initially, it is represented by a hypertrophic and uncontrolled explorative behaviour towards the door, the windows or the object with the owner's scent (Ozella & Pessani, 2015). An oral phase follows the olfactory phase. This second stage can be destructive, and the dog can demolish or ingests objects. The animal can show motor restlessness, walks in circle, shakes and trembles. Sometimes, on the contrary, it immobilizes itself and is unable to move. The dog can manifest gastrointestinal symptoms with sialorrhea, vomit, diarrhoea and involuntary urination (Horwitz, 2009).

The therapy with pheromone is adequate when the symptoms are mild. When the disorder is severe, a pharmacological long-term therapy with Serotonin Reuptake Inhibitor (fluoxetine, fluvoxamine) is indicated or, at least, a short-period medication with trazodone, a Serotonin Antagonist and Reuptake Inhibitor (SARI) (Ogi et al., 2018). When the patients are inhibited and do not communicates, it can be used the clomipramine. The therapy wants to reduce the level of anxiety and increase the sense of security when the dog is alone through desensitisation. It is necessary to reduce the dependence of the dog from the owner. It can be useful the teaching of orders, such as "rest" or "sit-down", that help the dog to remain calm away from its owner. Counterconditioning techniques can be employed to reduce the anxiety caused by the preparations before the separation-time (Blackwell, 2006; Giussani, 2016).

Primary Desocialization

The socialization leads to the acquisition of communicative system between the pup and its conspecifics and its heterospecifics. The socialization period occurs from the 3rd to the 12th weeks (Lindsay, 2000). During this period the mother represents a teacher for the puppies, and they learn self-controls and, for example, the submission posture or the alimentary hierarchy (Giussani, 2005; Dehasse, 2007; Giussani, 2007).

The orphaned puppies miss the educational action of the mother. If the caregiver is not well prepared, these subjects risk to not learn self-controls and the correct communicative codes, so they will became unable to control themselves and to communicate, and, potentially, aggressive. The symptoms can be aggression for irritation or for conflict situations, bite without threat, unable to submission, absence of social hierarchical behaviours, both nutritive and sexual (Confente, 2018).

Stereotypies and compulsive disorders

The stereotypes are repetitive and predictable motor patterns, acted without any apparent goal, which are shown in situations of conflict and frustration. The conflict arises when the subject has the motivation to product two opposite behaviours, such as when it is unsure on the result of an event or when the owner response incoherently to dog's behaviour. The dog has a motivation to act but it is hindered (Landsberg et al., 2013).

When the dog has not of valid strategies to face a situation, it can act substitution activities or redirected behaviours. However, when the situations of conflict are repetitive and persistent, the stereotypies can be shown habitually and can progress in compulsive behaviours (Landsberg et al., 2013, pp. 164-174). These behaviours are anomalous, repetitive, exaggerated behavioural sequences and are the aberration of normal behavioural modules (grooming, predation, ingestion and locomotion). Therefore, they are shown self-traumatism, alopecia, tail chasing, pica and pacing (Tiira et al., 2012; Overall, 2013) and self-direct aggression (Ruffoni, 2018). Another compulsive behaviour, with a hallucinatory component, is the fly biting (Landsberg et al., 2013).

Stereotypies represent a mean to respond to a stressful situation and achieve an equilibrium. Instead, the compulsive behaviours interfere with the normal vital functions. The lack of the mother can be the cause of the development of the stereotypies for the frustration of the failure to satisfy the motivation to suckle or for insufficient social contact (Landsberg et al., 2013). The confinement in hypo stimulant environment can be another cause of this kind of behavioural disorders (Lindsay, 2000).

Anomalies of transmission of serotonin are identified as etiopathogenic mechanism. The therapy is based on offering to the animal social and environmental enrichments, such as play activities and interactions. In severe cases, it can be necessary a pharmacological long-term therapy (fluoxetine, clomipramine, anxiolytics and antiepileptics) (Landsberg et al., 2013).

Conclusion

Understanding how domestication and socialization influence the dog allows the human comprehension of this species and helps to improve the relationship between human and dog and to prevent or manage behavioural problems. The caregiver must survive the puppy and ensure it to grow up with an emotional stability. The people that decides to take care of orphaned puppies must have the right competences and know that the puppy have physical and psychological needs, in order to prevent behavioural disorders in the adult dog.

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La cura psicologica ed emozionale del cucciolo orfano: management e rischi di disturbi comportamentali

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Sintesi

Sotto la guida esperta ed amorevole della madre che sa come educare le sue esperienze di gioco, il cucciolo cresce emotivamente bilanciato, impara ad essere autonomo, a controllare la sua esuberanza e a relazionarsi correttamente con i conspecifici ed ad animali.

Se la madre è assente, un essere umano deve prendersi cura del cucciolo orfano. Colui che si prende cura del cucciolo deve assicurarsi che esso sia sano non solo fisicamente ma anche da un punto di vista psicologico, in modo da svilupparsi in un soggetto adulto equilibrato.

La persona che decide di prendersi cura del cucciolo orfano deve essere a conoscenza del comportamento materno canino e dovrebbe cercare, nei limiti del possibile di emularlo.

Gli studi sui periodi sensibili hanno permesso di identificare quelli in cui, durante la crescita del cucciolo, l'esposizione ad uno stimolo specifico produce il massimo effetto.

Il periodo di socializzazione è sicuramente quello in cui l'esposizione a determinati stimoli produce effetti più incisivi.

La persona che si occupa del cucciolo deve dare all'animale la possibilità di conoscere conspecifici ed eterospecifici, assicurando ad esse uno stile di vita ricco di stimoli.

Nonostante la buona volontà delle persone, l'azione di cura di un cucciolo può presentare carenze ed errori. È comunque essenziale che la persona aiuti il cucciolo nella sua maturazione psicologica in modo da prevenire l'insorgenza di disturbi comportamentali.

È soprattutto importante una corretta gestione del processo di attaccamento e di distacco per evitare lo sviluppo di paure, ansia, fobie e di quelle sindromi che sono causate da un'alterazione dell'omeostasi sensoriale, come la sindrome da privazione sensoriale, la sindrome di ipersensibilità-iperattività, la desocializzazione primaria, le stereotipie ed i disordini compulsivi, l'aggressività e la depressione.

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A case of polydipsia and inappropriate urination in a mongrel dog

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Abstract: A 10-year-old mongrel castrated male dog, weighting 16 Kg, was examined for polydipsia and inappropriate urination.

Polydipsia occurred at stressing events / periods or for apparent boredom, while inappropriate urination occurred even without clear external stimulation, in various areas of the home and at different times of the day and week.

The laboratory and ultrasound findings allowed to exclude a medical cause. The diminishing of polydipsia following behavioral interventions, allowed to diagnosticate a psychogenic polydipsia.

It was explained to the owners that the dog should not be punished when he carries out the undesirable behavior. It should rather identify when the dog is going to drink in order to anticipate and involved him in other activities The owner was instructed to prevent the stressful and conflict situations. A food supplement based on L-theanine (Anxitane®) for one month (4.5 mg/Kg BID for the first 7 days then 3 mg / Kg BID) was recommended.

Two weeks after the first visit and during the following months, there were no more episodes of polydipsia, so the water restriction program was not undertaken.

During this period, two episodes of excitement/emotional urination took place. It was decided to associate with Anxitane®, Adaptil® collar for one month.

Four months after the visit the owner reported that the dog apparently had less control over the urinary sphincter. To date, the dog is not following any nutraceutical or pharmacological treatment, and the situation is under control (two, three episodes per month)

Key Words: dog, polydipsia, inappropriate urination

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Presentation

A 10-year-old mongrel castrated male dog, weighting 16 Kg, was examined for polydipsia and inappropriate urination.

Polydipsia occurred during stressing events / periods or because of apparent boredom, while inappropriate urination occurred even without clear external stimulation, in various areas of the home and at different times of the day and week. Both problems were cyclical.

History and presenting signs

The dog was found on a beach at the age of 7 months, approximately. No information was available on the behavior of parents and siblings, on the age of weaning, nor on the type of stimulation (people, other animals, places and situations) that he faced in the period prior to adoption.

The dog lived with the two owners (both workers: one full-time and one part-time) and a

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castrated male cat of 13 years (already present in the house upon arrival of the dog) in a large apartment with a terrace; he had full access to the rooms and the terrace in the presence of the owners.

He had an excellent relationship with both owners and the relationship with the cat had always been friendly. On weekdays, the dog stayed alone at home in the morning; on the weekend both owners were present.

The dog ate dry food, twice a day, at set times and homemade products (lettuce, carrots, bread) as a snack between meals. The appetite was always voracious and indiscriminate.

During stressful periods the water intake increased, leading to the consumption of 100-150 ml/kg/day; vice versa, in periods of remission, the daily intake corresponded to the needs of species, and did not exceed 80 ml/kg /day (Nelson, 2015).

The owner reported that the main trigger for the polydipsia was the arrival of guests, especially if numerous, moreover, the behavior seemed to be related to boredom.

About strangers, the dog assumed, at first, avoidance behaviors (moving backwards/away, looking away), displacement behavior (licking the truffle, yawning, chewing empty) (Landsberg et al., 2013d) and exhibited a tense posture (low head, backward ears, tail perpendicular to the floor). These manifestations were accentuated if the stranger approached abruptly the dog from above to pet him. This approach always caused an overreaction with snap of the head downwards, "whale eye" and muscle stiffness. Conversely, if they proposed themselves calmly and politely, using a calm tone of voice and avoiding imposing approaches, the dog was disposable to interaction and sometimes required pampering and attention, without, however, relaxing: the mouth, for example, always remained closed.

The dog usually urinated and defecated outdoors during walks (three times a day). The exploratory behavior was proportionate to the environmental stimulations. When he started to urinate at home too, it was evident an increase in daily frequency and volume and a change in the urinary behavior: sometimes the dog assumed a low and static posture and looked at the owners with a worried expression; at other times, it seemed that he was not able to hold the urine and that he lose it while he was walking, leaving a "trail" behind him.

A typical situation that frequently resulted in inappropriate urination consisted in trying to interact with both owners, who, however, were engaged in different activities; after having looked insistently at the owners, the dog exhibited an a-finalistic motor activity (mostly pacing) and, at the same time, unintentional urination. Inappropriate urination occurred both in the presence and in the absence of the owners but was more frequent in their presence.

Polydipsia always occurred in conjunction with inappropriate urination, which also occurred alone. Both symptoms followed an intermittent course since the dog was one year old, but at the time of the visit, critical periods were not distinguishable from normal ones.

If the dog exhibited these undesirable behaviors, the owners ignored or distracted him.

The dog showed regular self-grooming and occasionally licked his limbs for short time without lesions of any kind. He slept in different areas of the house, including the owners' bedroom, and regularly used a confined rest area where he went spontaneously and willingly and at the request of the owners.

The dog showed little interest in the play in general and towards objects, preferring short physical interactions with the owners. Aggressive behavior was exhibited, in the form of barks, only when he met other dogs.

The medical history included regular vaccination and parasiticidal prophylaxis. For about 4 years, the dog had modest tenderness in the hind limbs, which was detectable only during palpation; for this reason, he cyclically took an integrator that supports joint function (NBF LANES Artikrill®).

The dog was castrated at the age of one year and a half, attempting to reduce the inappropriate urination but without success. It was thus decided to undertake a drug therapy based on

clomipramine: this second intervention also did not produce the desired results (the owner also reported that he had not identified any side effects). The drug intake was suspended shortly after the beginning of a behavior modification course; during this time, inappropriate urination and polydipsia were in remission and the dog trainer focused only on the problem of barks. In the following years, the main problem came back with the same cyclical trend and an attempt was made to keep it under control by using numerous complementary feeds, with no noteworthy results.

Examination and laboratory exams

The physical examination was conducted in the veterinary clinic where the dog owner worked.

The dog was in good health condition and his BCS was estimated 6/9.

At the same time a behavioral observation was conducted: the dog was shy, wary and hesitant.

He was circumspect towards social stimuli and not being able to copy with them, he maintained a constant state of alert and concern. The owner reported that even at home he rarely relaxed.

The dog showed a marked olfactory exploration behavior, when he was not exploring, he was often owner-oriented. He promptly responded to owner's call and he was effectively reassured by visual and physical contact with him. The dog had learned to go to an area prepared with a mattress every time the intercom of the clinic rang. During the consultation the low motivation to play and the high interest in food rewards were confirmed.

Laboratory and imaging tests were performed to rule out medical reasons related to PU/PD. The results of the CBC were normal, while the biochemical profile revealed an increase in ALT enzyme (138 IU/ l, with reference range 10-60). The coprological examination for flotation was negative. Urinalysis showed a specific weight at the lower limit of the isosthenuric range (1008 g/l) (Di Bartola & Westropp, 2015; Skeldon & Ristic, 2017).

After excluding an inflammatory state, the diagnostic procedure was deepened through the dosage of urinary cortisol and an abdominal ultrasound: both tests were negative. The execution of a modified water restriction test (Bulliot & Hébert, 2016) was evaluated, taking into account its potential inapplicability: polydipsia, in fact, especially if prolonged, can reduce the hyperosmolarity of the interstice medullary (cd wash-out) with consequent inability to concentrate urine in response to water deprivation (Fanton et al., 1988; Schoeman, 2008; Taylor et al., 2010; Long et al., 2015; Nelson, 2015; Skeldon & Ristic, 2017) and, in these cases, testing serum osmolality becomes a more reasonable option (Feldman, 2007). The deprivation test was not actually performed, because the polydipsia, from intermittent, entirely stopped in the next 10 days.

Diagnosis

Increased water intake and urine production are more frequently due to medical causes (Nichols, 2001; Landsberg et al., 2013c; Nelson, 2015). An amount of water between 80 and 100 ml/kg/day suggests the possibility of polydipsia, while an intake of more than 100 ml/kg/day confirms it (Nelson, 2015; Skeldon & Ristic, 2017). Animals suffering from these disorders could present the low urinary specific weight as the only laboratory anomaly (Nichols, 2001).

In the case in question, both the requirements were ascertained; in fact, during critical periods, the intake of water exceeded 80-100 ml/kg/day and the specific urinary weight was at the minimum limit of the isosthenuric range. This calculation must be carried out at home (Behrend, 2005; Bruyette, 2015).

After having ascertained the presence of polyuria and polydipsia, the laboratory and ultrasound findings allowed to exclude the most probable medical causes: hyperadrenocorticism, diabetes mellitus, liver failure, chronic renal failure, pyelonephritis, hypokalemia, hypercalcemia, hypoadrenocorticism and acquired nephrogenic diabetes insipidus secondary. Central diabetes and psychogenic polydipsia were therefore investigated, because the biochemical screening tests showed no abnormalities during these rare conditions (Landsberg et al., 2013c; Nelson, 2015). At this point of the diagnostic procedure, however, the polydipsia began to decrease in frequency until it disappeared, probably for the effect of the behavioral therapy associated with the nutraceutical.

The first behavioral interventions, which decrease immediately the polydipsia, allowed to diagnosticate a psychogenic polydipsia, sign of a displacement behavior and/or attention seeking behavior. A displacement behavior is defined as a response that is apparently out of context (but normal in itself), capable of unloading the tension generated by a situation of conflict and frustration (Landsberg et al., 2013d). The attention seeking behavior can occur when the dog is fearful, anxious or participates in inconsistent interactions, allowing him to obtain information from the environment and, at the same time, be reassured by the owner; the latter can also reinforce it involuntarily, increasing its emission frequency (operant conditioning) (Overall, 2013a; Denenberg, 2018).

The fact that the behavior was exhibited mainly in the presence of the owners, excluded a separation problem, which implies the absence (real or perceived) of family members (Landsberg et al., 2013d; Overall, 2013a; Horwitz, 2018).

Some reservations need to be maintained in the interpretation of primary polyuria, given that in humans it has been shown that chronic hyperhydration regulates the release of vasopressin in response to hypertonicity (Rijnberk, 2007) and that also in dogs with primary polydipsia there are indications for abnormalities in the release of this hormone: a study by Van Vonderee et al. (1999) has shown that in some dogs with primary polydipsia there may be a non-linear relationship between vasopressin and plasmatic osmolality.

In this case, inappropriate urination was related to both psychogenic polydipsia (Tynes, 2018) and other causes, considering that it also occurred when drinking was normal.

Other differential diagnoses include: "submission"/conflictual urination; excitation urination and separation distress (Landsberg et al., 2013e; Overall, 2013a; Tynes, 2018).

Treatment

Regarding polydipsia, it is advisable to limit water intake reasonably, taking into account the chronic nature of this behavior. The reduction in recruitment to acceptable volumes must, in fact, be attempted over many weeks, in order to help the dog to establish alternative coping strategies while behavioral and pharmacological therapies are carried out (Landsberg et al., 2013c).

The owner was asked to calculate, approximately, the water consumption in 24 hours and to reduce it by 10% every week (Nelson, 2015), by dividing the daily volume into numerous administrations (Nelson, 2015) or with the use of a modified bowl that reduces water intake times (for example, DrinkBetter®); both possibilities are feasible only if the dog is already acquiring new coping strategies with other therapeutic aids, because otherwise the problem could get worse.

To counteract the reduced medullary hyperosmolarity, it was recommended to orally administer cooking salt (1g / 30 kg every 12 hours) for 3-5 days (Nelson, 2015).

It was explained to the owners to not punish the dog when he carries out the undesirable behavior, because punishment could increase anxiety and fear, as well as not teaching him any-

thing new (Landsberg et al., 2013c, d; Sueda, 2018). It should rather identify when the dog is going to drink (even using a diary), in order to anticipate him and involved him in other activities (social interactions, play, exercises), rewarding him for the activity; in this way, undesirable behavior is redirected towards an alternative action, such as "Come!"/ "Look!"/ "Sit!" (Landsberg et al., 2013c).

Regarding inappropriate urination, the owner was instructed to prevent the situations generating conflict and stress. When exposure cannot be avoided, the owner was explained how to weaken stressful social stimuli, focusing on the interactions with the owners and managing the encounter with strangers. Finally, it is advisable to keep the bladder as empty as possible (Overall, 2013a), so it was suggested to increase the walks before predictable stressful/exciting events.

Concerning the anxiety, it was recommended to reinforce any state of calm, enrich the environment (for mental and physical stimulation) and create a routine that was as predictable as possible (Landsberg et al., 2013c, d; Sueda, 2018). The owner decided to take the dog at his work place, a choice that gave several advantages: more opportunities to urinate out; more time spent together with the owner; daily routine more stable, at least on weekdays.

Since the relationship with the owners was already optimal, they were only advised to try to maintain a calm attitude when the dog showed the undesirable behavior, to avoid increasing their negative emotional state (Palagi et al., 2015; Albuquerque et al., 2018; Sinischalchi et al., 2018).

Daily exercises (sessions of 10-15 minutes) were recommended. In particular we suggested the use of Kong® Senior, with initial low level of difficulty and in controlled situations (quiet and familiar areas and under the supervision of the owner, so as to be able to verify the reactions and evaluate the right moment to leave the dog with the object), mentally stimulating activities with the owner and problem-solving activities (Landsberg et al., 2013a).

When the dog was alone at home, it was advised to muffle external noises with the help of white noises (Landsberg et al., 2013d) or relaxing CDs (Wells et al., 2002; Boone & Quelch 2003), not before having carried out tests to exclude that they did not create stress to the animal.

Finally, the use of a food supplement based on L-theanine (Anxitane®) (for one month 4.5 mg/Kg BID for the first 7 days, then moving to 3 mg/Kg BID) was recommended. Several studies have suggested that this nutraceutical is effective in reducing behavioral responses related to anxiety and fear (Dramard et al., 2007; Araujo et al., 2010; Pike et al., 2015).

Follow up

Two weeks after the first visit and during the following months, there were no more episodes of polydipsia, so the water restriction program was not undertaken. Daily intake of water (with free access to the source) settled on 70 ml/kg/day and urinary specific gravity, measured on samples collected at different times of the day for greater reliability (Nelson, 2015; Skeldon & Ristic, 2017), remained stable between 1015 and 1017 g/l, in conditions of normal hydration. These values were curiously confirmed even after an empirical water restriction at 3 and 6 hours, when in a healthy subject we would expect differently; this apparent inability to concentrate the urine would support the thesis that animals suffering from polyuria and polydipsia, suffering from the loss of solutes in the renal medulla, tend to produce relatively dilute urine (Fanton et al., 1988; Schoeman, 2008; Taylor et al., 2010; Long et al., 2015; Nelson, 2015; Skeldon & Ristic, 2017). Oral salt administration did not change the situation.

During the first month after the visit, guests were not invited into the house; the weekly routine was regularized, except for the weekend; once a week nose work were performed with the owner; the biweekly Kong® activity turned out to be enthusiastic. During this period, two episodes of excitement/emotional urination took place: one, with attached defecation, as a result of a chase to a blowfly; the second, after playing with the Kong®. In this regard, it is advisable to

refine the interaction with the object, bringing the dog to urinate before interacting with the Kong® and avoiding removing the object quickly after it has been emptied, either because this could be interpreted as a negative punishment, both because after a first engaging and exciting interaction linked to the consumption of food (highly euphoric for the dog), a second, more bland interaction could help to diminish the arousal in a more natural way.

Two months after the visit, sporadic episodes of conflict urination occurred, almost exclusively during the weekend, when it was easier for both owners to be present and because the regularity of the weekly routine disappeared. In this regard, it was very useful, but not always enough, to involve the dog in alternative activities.

Anxitane® at initial dosage (4.5 mg/kg BID) was prescribed for few days before stressful events.

Three months after the visit, the episodes of inappropriate urination during the weekend increased, although remaining below the average. Answer replacement exercises and nose work were no longer implemented, but Kong® was maintained 1-2 times a week. It was decided to associate with Anxitane®, Adaptil® collar for one month. The use of the collar did not yield results worthy of note and was therefore not reapplied the following month. The owner also decided to suspend Anxitane®.

Four months after the visit, the situation had not changed much: occasional urination from excitement in different environments and conflict urination/"submission" almost exclusively at home during the weekend. The owner, however, reported that the dog apparently had less control over the urinary sphincter. For this specific problem there are two possible molecules: phenylpropanolamine, which can help in cases of "submission" and excitement urination if a poor sphincter competence is a concurrent factor (Landsberg et al., 2013b, e; Tynes, 2018), and clomipramine which, given the cardiocirculatory side effects of phenylpropanolamine and the established anxious component of undesirable behavior, would be more appropriate in this case, since it would produce both anxiolytic and moderately anticholinergic effects by increasing the urethral sphincter tone (Landsberg et al., 2013b). Furthermore, clomipramine was taken in the past without any adverse effects.

Conclusion

To date, the dog is not following any nutraceutical or pharmacological treatment, and the situation is under control (two to three episodes per month) with behavioral and environmental management. The owner is open to the possibility of undertaking drug treatment in the future.

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Un caso di polidipsia e di urinazione inappropriata in un cane meticcio

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Sintesi

Un cane meticcio, castrato, di 10 anni e 16 Kg di peso è stato esaminato per polidipsia e urinazione inappropriata.

La polidipsia si manifestava in eventi stressanti o di noia apparente, mentre l'urinazione inappropriata avveniva anche senza una chiara stimolazione esterna, in diverse zone della casa e in momenti diversi del giorno e della settimana.

I risultati degli esami di laboratorio e ecografici permisero di escludere una causa organica. L'intermittenza della polidipsia, insieme al fatto che era rapidamente diminuita in seguito ad un intervento esclusivamente comportamentale, ha permesso di diagnosticare una polidipsia psicogena.

Al proprietario fu chiesto di calcolare, approssimativamente, il consumo di acqua nelle 24 ore e di ridurlo del 10% ogni settimana.

È stato spiegato ai proprietari che il cane non dovrebbe essere punito quando si emette comportamenti indesiderati poiché ciò può aumentare l'ansia e la paura, oltre a non insegnargli nulla di nuovo.

Si dovrebbe invece individuare le situazioni che inducono il cane a bere (anche usando un diario), in modo da anticiparlo e coinvolgerlo in altre attività (interazioni sociali, gioco, esercizi), premiandolo; in questo modo, il comportamento indesiderato è rediretto verso un'azione alternativa, come "Vieni", "Guarda", "Seduto".

Il proprietario è stato educato a prevenire, quando possibile, le situazioni che generano conflitto e stress. È stato suggerito l'uso del Kong®Senior, inizialmente con un basso livello di difficoltà e in situazioni controllate.

È stata inoltre raccomandato di integrare la dieta con L-teanina (Anxitane®) (per un mese 4,5 mg/Kg BID per i primi 7 giorni, quindi 3 mg/Kg BID).

Due settimane dopo la prima visita e nei mesi seguenti, non ci furono episodi di polidipsia, per cui la restrizione idrica non fu applicata.

Nel primo mese dopo la visita, non furono invitati ospiti a casa; la routine settimanale era regolare, ad eccezione del week-end; una volta a settimana erano effettuati giochi di fiuto con il proprietario. Il gioco con il Kong, bisettimanale, era molto gradito dal cane.

Durante questo periodo, si verificarono due episodi di urinazione emozionale o da eccitazione. Si decise quindi di associare all'Anxitane, l'Adaptil collare, per un mese. L'uso del colare non diede risultati e perciò non fu applicato nel mese successivo. Il proprietario decise anche di sospendere l'Anxitane.

Quattro mesi dopo la visita la situazione non era cambiata: si verificavano episodi di urinazione da eccitazione in differenti ambienti e urinazione da "sottomissione" o da conflitto, quasi esclusivamente a casa durante il weekend.

Il proprietario riferiva l'impressione che il cane avesse un minor controllo dello sfintere uretrale. Ad oggi il cane non è sottoposto a trattamenti farmacologici o con nutraceutici e la situazione è sotto controllo (2-3 episodi al mese) in seguito a gestione comportamentale ed ambientale. Il proprietario è disponibile alla possibilità di sottoporre il cane, in futuro, ad un trattamento farmacologico.