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The dog (*Canis familiaris*) as part of the family: a pilot study on the analysis of dog bond to all the owners

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Abstract: The aim of the current study was to evaluate whether dogs living in multi-member families show a stronger bond towards a specific person, and, if it is the case, which characteristics of the owner or of their relationship may lead to such preference.

Eleven dogs were tested using a modified version of Ainsworth Strange Situation Test where all the family members (five 2-member, two 3-member, and four 4-member families) were contemporaneously present. The duration of 19 non-social (proximity to door/chair, behaviors towards door/chair/shoe, oriented to door, exploration, locomotion, passive behavior, individual play, vocalisations, and whining) and social (attention seeking, physical contact, following, proximity, approach, and visual orientation) dog behaviors was measured. The latter were assessed towards each participant. A questionnaire, including the Lexington Attachment to Pets Scale, was used to gather information on the relationship between people and the tested dog.

The analysis of data revealed that most dogs (n = 8; 72.7%) living in a multi-member family show a stronger bond to a specific member. Owners usually (75.0%) were able to identify the person the dog was more bonded to. It was not found a correlation between the level of attachment of a specific owner to the dog and the bond of this dog to that specific owner (57.12 ± 15.42 *versus* 58.00 ± 18.95; F = 0.00; p = 0.955). Among dogs who showed a preference, the majority (n = 6; 75.0%) preferred people who managed the dogs almost totally by themselves; for the remaining two, the preferred person was the one within the family who dealt with walking and food or walking and play. Therefore, walking the dog seems to increase the likelihood of establishing a strong bond with the dog.

Key Words: Ainsworth Strange Situation test, behavior, bond, dog, family, owner.

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Introduction

In the 1950's John Bowlby (1988), starting from psychoanalytic concepts and ethology, elaborated a theory of attachment that was valid for all mammals. Attachment bonds, defined as an affectional tie enduring over time, is formed by one person or animal between himself and another specific one (Ainsworth & Bell, 1970). The behavioral test commonly used to study the child's attachment to the mother is called Ainsworth's Strange Situation Test (ASST) (Ainsworth & Bell, 1970).

Canis familiaris is a highly social species. As the ecological niche of domestic dogs is the human social environment, a variety of studies concerning dog behavior in relation to humans were developed. Topàl and colleagues (1998), assuming that dog-owner relationship resembles child-caregiver one, were the first who analysed the dog-human bond as an attachment by using a modified version of the ASST. Adult dog's behavior during the separation from the owner as well as upon their reunion suggested that the ASST is suitable to provide information regarding the dog-human relationship.

Few studies have investigated dog-dog attachment using the ASST (Mariti et al., 2017; 2014).

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Research has instead focused on the relationship between a dog and a specific person, namely the owner (for a review see Payne et al., 2015). There is now scientific evidence that adult dogs can use their owner as a secure base (Mariti et al., 2013), and they can form new interspecific attachment bonds even after the breaking of previous ones (Gácsi et al., 2001). However, many dogs live in multi-member families, and they relate to all members of the fostering family.

The aim of the current study was to evaluate whether dogs living in multi-member families show a higher attachment to a specific person, and, if it is the case, which characteristics of the owner or their relationship may lead to such preference.

In order to do that, all dogs were tested using a modified version of ASST where all the family member were contemporaneously present. Besides participating in the behavioral test, each family member filled in a questionnaire to gather information on his/her relationship with the examined dog.

Materials and methods

Subjects

Eleven families and their dogs participated in the experiment. The families were all volunteers and they were recruited by personal contacts.

Dog owners were 19 women and 13 men, whose ages ranged from 7 to 61 years.

Table 6, 7 and 8 report the characteristics of families and family members participating at this study.

Dogs were 9 females and 2 males, whose age varied from 13 to 108 months. They were: 3 Labrador Retrievers, 1 Border Collie, 1 Australian Shepherd, 1 Dalmatian, 1 Beagle, 1 Pug, 1 Miniature Poodle, and 2 mixed-breeds. None of the female dogs were in oestrus, nor were they pregnant at or around the time of testing. The inclusion criteria for tested dogs were: being more than 12 months old, having lived with the families for more than 6 months, being used to a wide variety of different environments and people (which meant no marked fear or aggression to strangers, for safety reasons).

Experimental setting

The experimental environment was a relatively bare room, unfamiliar to the dogs, at the Department of Veterinary Sciences - University of Pisa (Italy). The room (4.50 x 4.30m) was prepared to match as much as possible the requirements described in the Ainsworth Strange Situation Test, especially in its version modified for dogs. The room was equipped with: chairs, in semicircle, in a number equal to participants (all the family members plus the stranger); a water bowl; three dog toys (a ball, a puppet and a rope); a table to leave the leash on; one door, around which it was drawn a 1 meter radius semicircle; two videocameras (JVC® GZ-MG 130E) to record the whole test. One videocamera was oriented to the door and the surrounding area, while the other one recorded the whole room.

Procedure

Procedures used until now for the study of dog attachment to people slightly differed one from another. For the specific aim of the current research, some changes were made, especially related to the number of people participating at the same time.

All the family members and a stranger participated contemporaneously in the test. The stranger was always played by the same woman, who had never met the dog before. The stranger also acted

as test leader, guiding other participants throughout the test; when the stranger was outside the room, instructions were given using a low voice from outside.

The participants were asked not to draw the attention of the dog and to remain seated during the whole test, except when they had to leave or come back in the room, and when they were asked to stimulate the dog to play.

All participants brought a shoe with them as a personal object. The shoe of each participant, before the experiment began, was put in a plastic bag on the chair of the person seated on the left of the shoe owner.

The entire procedure comprised two consecutive phases: pre-experimental phase and experimental phase, the latter divided into 5 episodes plus an introductory episode. Episodes 2 and 3 were repeated for each participant (all family member plus the stranger), therefore the total duration of the test varied from a minimum of 23'22", when participants were three (two family members plus the stranger), to a maximum of 30'45", when participants were five. The leaving order of the family members was decided randomly before the beginning of the test, except for the stranger, who was always the last person leaving the room.

Pre-experimental phase: all the family members were escorted to a waiting room and asked to fill in a questionnaire in order to gain background information on: characteristics of the dog and the environment where he/she was living, personal details of the respondent, kind of activity involving the respondent together with the dog, and the Lexington Attachment to Pets Scale (L.A.P.S.) (Johnson et al., 1992; Marinelli et al., 2007).

The procedure was briefly described to the family members before the test. The specific aim of this study was not disclosed until the end of the test, in order to avoid possible influences on the participants' responses and behavior; the owners were told that the study aimed at investigating the exploratory behavior of dogs in an unfamiliar environment.

Experimental phase:

Introductory episode: all family members, stranger and dog entered in the experimental room. Participants sat on the chairs as previously established. The dog was unleashed and set free to explore the room. The leash was laid on the table.

Episode 1: Family members, stranger and dog (3 min). Participants could talk to each other and interact with the dog only if the latter was seeking for their attention. At the end of the third minute a person left the room.

Episode 2: A participant was out of the room. First minute: participants had to ignore the dog, even if he/she was seeking for attention, and they could not talk to each other. Second minute: the person who sat on the left side of the one who had left the room, pulled out the shoe from the plastic bag and put it on the empty chair. Participants could talk to each other and only interacted with the dog if he/she was seeking for attention. Third minute: the person seated on the right side of the one who had left the room tried to stimulate the dog to play, with a maximum of three trials (one for each toy in the room). As soon as the dog started playing or at the end of the third trial, the stranger declared the end of the episode and called the person to come back into the experimental room.

Episode 3: The participant came back into the room. First minute: the person who was outside the room knocked on the door and stayed behind it for 10 seconds. At the end of that, the person entered the room and stayed for 50 seconds within 1 meter from the door, to allow the dog greeting him/her. If the dog initiated interaction, the person greeted and comforted the animal as he/she usually did when returning at home; if the dog did not approach the person, he/she had to wait the end of 50 seconds close to the door, without drawing the dog attention. Meanwhile the other participants could not speak nor interact with the dog. Second minute: the person who had just entered took the shoe off the chair and sat. Participants could talk to each other and interact with the dog only if the animal was seeking for their attention. Third minute: the person who came back in the room tried to stimulate the dog to play with a maximum of three trials (one for

each toy present in the room). As soon as the dog started playing or at the end of the third trial, the stranger claimed the episode was finished and another participant left the room.

Episode 4: Dog alone. After episode 3 was repeated even for the last person who had left the room (the stranger), all the participants simultaneously left the room. The dog was left alone in the room for 1 min.

Episode 5: All the participants came back into the room. If the dog initiated interaction with a person, he/she could greet the dog as they usually did returning home. The participants sat in the same chairs as before and made conversation, interacting with the dog only if the dog was seeking for attention. The episode 6 lasted 1 minute.

At the end of each test, the experimental room's floor was washed using a non-toxic, weakly scented disinfectant.

Analysed behaviors

The eleven videotaped tests were analysed recording the duration (in seconds) of 19 behaviors divided into social and non-social behaviors (tables 1 and 2); each social behavior was analysed towards all the family members and the stranger. Analysed behaviors are listed in tables 1 and 2, accompanied by relative definitions and references.

Table 1. Non-social canine behaviors recorded in the Ainsworth Strange Situation Test.

Behaviors	Definition	Relative references
Exploration	Activity directed toward physical aspects of the environment, including sniffing, close visual inspection, distal visual inspection, and gentle oral examination such as licking	Mariti et al., 2013
Locomotion	Walking, pacing or running around without exploring the environment nor playing or following	Modified from: Prato-Previde et al., 2003
Passive behavior	Sitting, standing or lying down without any obvious orientation toward the physical or social environment	Topàl et al., 1998; Prato-Previde et al., 2003
Individual play	Any vigorous or galloping gaited behavior directed toward a toy when clearly not interacting with any participants; including chewing, biting, shaking from side to side, scratching or batting with the paw, chasing rolling balls and tossing using the mouth	Mariti et al., 2013
Proximity to the door	The time spent close to the door (<1 m) regardless to gaze orientation	Mariti et al., 2013
Behaviors towards the door	All active behaviors resulting in physical contact with the door, including scratching the door with the paws, jumping on the door, pulling on the door handle with the forelegs or mouth	Mariti et al., 2013
Oriented to door	Staring fixedly at the door, either when close to it or from a distance	Mariti et al., 2013
Behaviors towards the chair	All active behaviors resulting oriented to a family member's or stranger's empty chair	Mariti et al., 2013
Proximity to the chair	The time spent close to the empty chair	Current study
Behaviors towards the shoe	All behaviors resulting oriented to the shoe during shoe's owner absence, including staring the shoe, biting, shaking, dragging, sniffing	Mariti et al., 2013

Vocalizations	Barking, growling, howling... (excluding whining)	Modified from: Prato-Previde et al., 2003
Whining	Whining	Palestrini et al., 2010
Other behaviors	Any activity not included in the behavioral catalogue, such as self-grooming, self-scratching or drinking	Prato-Previde et al., 2003; Palmer & Custance, 2008

Table 2. Social canine behaviors recorded in the Ainsworth Strange Situation Test.

Behaviors	**Definition**	**Bibliographic references**
Attention seeking	Seeking attention from a person to play, be petted etc	Mariti et al., 2013
Physical contact	Being in physical contact with a family member or the stranger	Mariti et al., 2013
Following	Following the person around the room or to the door	Mariti et al., 2013
Approach	Moving towards, while clearly visually oriented to, a person	Mariti et al., 2013
Oriented to person	Staring fixedly (for a minimum 0.5 s) at a family member or the stranger, regardless of whether the behaviors was reciprocated	Mariti et al., 2013
Proximity	Close to (not in physical contact) a family member or the stranger at least for 3 seconds	Mariti et al., 2013

Statistical analysis

The statistical analysis has been carried out on each single dog, comparing the behavior displayed by an individual dog towards/in the absence of each familiar person and the stranger.

Some behaviors have been grouped in order to create the following behavioral categories:

- contact/proximity (referred to each participant), formed by: attention seeking, physical contact, following, proximity, approach, visual orientation;
- door/chair/shoe, formed by: proximity to door/chair; behaviors towards door/chair/shoe; oriented to door.

Although behaviors grouped in categories have the same meaning, they express a different degree respectively of maintenance contact effect and protest at separation. Based on the intensity of analysed behaviors, different weights (0 to 1) have been assigned to each of them. Subsequently, the time spent displaying each behavior has been multiplied for the corresponding weight; finally, all the values have been summed, obtaining an assessment of the time spent in the maintenance contact effect and protest at separation activities which considers the intensity of displayed behaviors.

The following factors of multiplication have been assigned:

- for the category called contact/proximity to a person:
  - contact: 0.5
  - attention seeking, following and proximity: 0.2
  - approach, visual orientation: 0.1
- for the category called door/chair/shoe:
  - behaviors towards the door: 0.5
  - proximity to door and behaviors towards chair/shoe: 0.3
  - orientation to door/chair: 0.1

The statistical analysis was carried out by using the X^2 test ($p < 0.05$).

For each dog a score has been calculated as follow. If the value relative to a family member re-

sulted statistically higher than the value relative to at least another participant, to the first person was assigned a partial score of 1; if at least at one of the other participants corresponded a statistically significant difference compared to at least one of the remaining participants, to this person was assigned the score of 1 and to the first it was added 1 point of score. For each dog a table has been created, summarizing the results and the score obtained for each behavior/category as follow:

1. the score obtained summing the number of X^2 resulted statistically higher for a specific person towards other participants for: door/chair/shoe in episodes 2 (when that person was out of the room), contact/proximity to a person in episodes 3 (when that person re-entered the room), and contact/proximity to a person in episodes 6 (when all people re-entered the room);
2. the score regarding possible differences in the dog's greeting to each participant at his/her re-entering (calculated according to Topál et al., 1998);
3. the score regarding possible differences in the dog behavioral response to play stimulation by each participant;
4. the score regarding whining duration for episodes 2;
5. the total score obtained summing scores at point 1 to 4.

A participant has been considered as the preferred by the dog when his/her total score was higher than other participants' score. In case the higher score obtained by family members was equal or lower than the stranger's score, none has been recognized as preferred person for that dog.

Regarding the questionnaires, for the current study the following items have been take into account:

1. the management of the dog: who in the family dealt with food, walking, play and training;
2. the person who, according to the respondent, was considered as the preferred one by the dog;
3. the L.A.P.S. score, calculated as in Marinelli et al., 2007. Scores obtained by preferred and not preferred people were compared using ANOVA ($p < 0.05$).

Results

Tables 3, 4 and 5 report results obtained by the analysis of videos.

Table 3. Results obtained for dogs living in two-people families.

Dog and owners		DCS objects Ep. 2	CP person Ep. 3	CP person Ep. 5	Whining		Greeting	Social play	Total
A									
AO1	Duration	11.6	40.5	0.5	3	Score	1	2	3
	Points	1	2	0	0	Points	0	0	
AO2	Duration	0.0	38.3	0.3	3	Score	1	2	1
	Points	0	1	0	0	Points	0	0	
Stranger	Duration	0.0	17.9	0.7	1	Score	1	2	0
	Points	0	0	0	0	Points	0	0	
B									
BO1	Duration	15.5	40.1	9.0	6	Score	3	0	2
	Points	1	0	0	1	Points	0	0	
BO2	Duration	16.5	47.4	2.1	4	Score	3	0	2
	Points	1	0	0	1	Points	0	0	
Stranger	Duration	3.0	50.4	6.9	0	Score	3	0	0
	Points	0	0	0	0	Points	0	0	

C									
CO1	Duration	22.4	14.4	9.0	2	Score	3	2	2
	Points	1	0	1	0	Points	0	0	
CO2	Duration	20.7	16.5	5.9	6	Score	3	2	1
	Points	1	0	0	0	Points	0	0	
Stranger	Duration	0.0	12.3	0.7	1	Score	3	2	0
	Points	0	0	0	0	Points	0	0	
D									
DO1	Duration	14.7	14.6	8.6	20	Score	3	2	1
	Points	0	0	0	1	Points	0	0	
DO2	Duration	12.4	13.2	14.4	8	Score	3	2	1
	Points	0	0	1	0	Points	0	0	
Stranger	Duration	9.2	14.6	5.9	10	Score	3	2	0
	Points	0	0	0	0	Points	0	0	
E									
EO1	Duration	19.1	13.0	0.2	3	Score	1	0	1
	Points	1	0	0	0	Points	0	0	
EO2	Duration	2.0	10.8	9.4	0	Score	3	2	3
	Points	0	0	1	0	Points	1	1	
Stranger	Duration	0.1	14.6	0.2	0	Score	1	0	0
	Points	0	0	0	0	Points	0	0	

Table 4. Results obtained for dogs living in three-people families.

Dog and owners		**DCS objects Ep. 2**	**CP person Ep. 3**	**CP person Ep. 5**	**Whining**		**Greeting**	**Social play**	**Total**
F									
FO1	Duration	10.1	4.8	2.6	12	Score	1	2	1
	Points	0	0	0	1	Points	0	0	
FO2	Duration	19.9	1.8	0.4	12	Score	1	2	1
	Points	0	0	0	1	Points	0	0	
FO3	Duration	14.9	3.1	34.7	22	Score	3	2	4
	Points	0	0	1	2	Points	1	0	
Stranger	Duration	1.6	0.7	0.7	0	Score	1	2	0
	Points	0	0	0	0	Points	0	0	
G									
GO1	Duration	30.6	20.1	18.2	11	Score	3	2	5
	Points	2	1	1	0	Points	1	0	
GO2	Duration	17.3	1.4	6.1	42	Score	1	2	1
	Points	1	0	0	0	Points	0	0	
GO3	Duration	9.2	15.9	3.5	7	Score	1	2	1
	Points	0	1	0	0	Points	0	0	
Stranger	Duration	6.2	1.3	0.0	64	Score	1	2	0
	Points	0	0	0	0	Points	0	0	

Table 5. Results obtained for dogs living in four-people families.

Dog and owners		DCS objects Ep. 2	CP Person Ep. 3	CP person Ep. 5	Whining		Greeting	Social play	Total
H									
HO1	Duration	44.5	29.0	13.0	15	Score	3	1	3
	Points	1	1	0	1	Points	0	0	
HO2	Duration	41.9	33.0	0.0	8	Score	3	2	2
	Points	1	0	0	0	Points	0	1	
HO3	Duration	35.2	67.0	0.0	4	Score	3	1	1
	Points	1	0	0	0	Points	0	0	
HO4	Duration	40.3	44.0	5.0	9	Score	3	2	2
	Points	1	0	0	0	Points	0	1	
Stranger	Duration	17.0	158.0	27.0	0	Score	3	1	3
	Points	0	2	1	0	Points	0	0	
I									
IO1	Duration	45.9	19.7	21.4	4	Score	3	2	4
	Points	1	0	2	0	Points	0	1	
IO2	Duration	3.8	24.1	0.0	0	Score	3	2	2
	Points	0	1	0	0	Points	0	1	
IO3	Duration	4.1	11.1	0.0	0	Score	3	1	0
	Points	0	0	0	0	Points	0	0	
IO4	Duration	43.2	38.6	5.9	17	Score	3	2	6
	Points	1	2	1	1	Points	0	1	
Stranger	Duration	0.0	66.4	0.9	0	Score	3	2	4
	Points	0	3	0	0	Points	0	1	
L									
LO1	Duration	19.6	19.7	9.2	0	Score	3	0	4
	Points	1	1	1	0	Points	1	0	
LO2	Duration	2.7	5.5	0.0	0	Score	3	0	1
	Points	0	0	0	0	Points	1	0	
LO3	Duration	1.0	5.3	0.0	0	Score	1	0	0
	Points	0	0	0	0	Points	0	0	
LO4	Duration	46.9	40.4	2.1	4	Score	3	0	5
	Points	2	2	0	0	Points	1	0	
Stranger	Duration	2.6	5.9	0.1	0	Score	3	0	1
	Points	0	0	0	0	Points	1	0	
M									
MO1	Duration	9.3	19.7	0.2	1	Score	1	0	2
	Points	0	1	0	0	Points	1	0	
MO2	Duration	43.3	13.2	10.2	0	Score	1	0	4
	Points	1	1	1	0	Points	1	0	

MO3	Duration	37.6	11.1	0.0	0	Score	1	0	3
	Points	1	1	0	0	Points	1	0	
MO4	Duration	2.1	16.3	0.0	0	Score	1	0	2
	Points	0	1	0	0	Points	1	0	
Stranger	Duration	9.7	3.5	0.0	1	Score	1	0	0
	Points	0	0	0	0	Points	0	0	

Notes for the tables 3, 4 and 5:

DCS = category called door/chair/shoe as described in materials and methods; CP= category called contact/proximity in materials and methods.

Duration = duration assessed as described in materials and methods; for Whining duration in seconds.

Score = score assessed as described in materials and methods.

Points = for the columns where the duration is reported, points are calculated as the number of X^2 that differ between participants, as described in materials and methods; for the columns where the score is reported, points are calculated as any difference in the score obtained by each participant.

Total = total score obtained by each participants, calculated as the sum of the points in each column considered.

Table 6. Summary of total scores obtained in the behavioral test and data from questionnaires for dogs living in two-people families.

Dog	Family members	Family members	Management of the dog				L.A.P.S. score	Age owner	Gender owner	Preferred Person
			Food	Walking	Play	Training				
A	2	AO1	x	x	x	x	H (55)	29	M	+
		AO2	-	x	-	-	M (43)	33	F	-
B	2	BO1	x	-	-	-	H (58)	29	F	-
		BO2	x	x	x	x	H (58)	35	F	-
C	2	CO1	x	x	x	x	H (53)	33	F	+
		CO2	-	x	x	x	H (61)	33	M	-
D	2	DO1	x	x	-	-	H (59)	47	F	-
		DO2	-	-	x	x	H (63)	11	F	-
E	2	EO1	-	-	-	-	M (45)	31	M	-
		EO2	x	x	x	x	H (61)	32	F	+
F	3	FO1	-	x	x	-	H (62)	24	F	-
		FO2	-	-	x	x	H (54)	30	F	-
		FO3	x	x	-	-	H (39)	59	M	+
G	3	GO1	-	x	x	-	M (36)	31	M	+
		GO2	x	-	-	-	M (41)	61	M	-
		GO3	x	-	-	-	H (47)	54	F	-
H	4	HO1	x	x	x	x	M (36)	45	F	-
		HO2	x	x	x	x	H (43)	14	M	-
		HO3	x	x	x	x	M (39)	10	F	-
		HO4	x	x	x	x	M (43)	45	M	-
I	4	IO1	-	-	-	-	L (22)	46	F	-
		IO2	-	-	-	-	L (27)	16	F	-
		IO3	-	-	-	-	M (45)	7	F	-
		IO4	x	x	x	x	M (35)	47	M	+

L	4	LO1	-	-	-	-	M (44)	50	M	-
		LO2	-	-	-	-	H (58)	7	M	-
		LO3	-	-	-	-	H (63)	10	F	-
		LO4	x	x	x	x	H (56)	41	F	+
M	4	MO1	-	-	-	-	M (44)	49	M	-
		MO2	x	x	x	x	H (53)	10	F	+
		MO3	-	-	-	-	H (64)	8	M	-
		MO4	x	-	-	-	H (56)	42	F	-

Notes for the tables 6:

For the L.A.P.S.= it was reported the level of attachment (H: high, M: medium, L: low) and the corresponding score calculated as Marinelli et al., 2007.

For owner' gender: M: male, F: female.

Regarding exploration, each dog showed its own trend in exploring the room. Overall, a strong reduction was observed between the episode 1 and 2.1, but no statistical difference was found between the duration of exploration when comparing episodes in which a certain participant was present versus when he/she was absent. In addition, no difference was observed in the comparison of episodes in which one participant versus another one were present. A trend of increased exploration emerged when the preferred person (found as described in tables 3, 4 and 5) re-entered the room, regardless of the order owners left the room: even in case the preferred person was the fourth leaving the room, the dog explored more after that family member was back.

Also concerning individual play, each dog displayed it in his/her own way (e.g. some of them did not play at all), and statistically significant differences were not found comparing presence to absence of each participant and comparing the presence of each participant to the others. But a trend of increased individual play was observed when one or more owners were present compared to their absence.

The analysis of behaviors such as locomotion, passive behaviors and vocalisations did not lead to relevant results.

Data elaborated through the analysis of videos, as well as data obtained by questionnaires, are reported and summarized in table 6.

The analyses revealed that 8 dogs out of 11 (72.7%) showed a preference for a person within the family. Among the remaining three pets, two of them lived in a two-people family (dogs B and D) and the third in a four-people family (dog H, for whom the higher total score obtained by an owner was equal to the total score of the stranger).

Considering dogs that showed a preference for a family member, 4 were more bonded to a female (on 13 women) and 4 to a male (on 11 men): therefore, the gender of people was not an important factor (X^2=0.043; p=0.835). Regarding the age, it did not emerge a preference for a specific range, as dogs show a stronger bond for people ranging from 10 to 65 years old.

Matching data obtained through questionnaires and behavioral tests, owners usually (24 on 32; 75.0%) seemed to be able to identify the person the dog was more bonded to.

It was not found a correlation between the level of attachment of a specific owner to the dog and the preference of this dog to that specific owner. As a matter of fact, considering only the owners of dogs who showed a preference (n=8), no difference was observed regarding the L.A.P.S. score between preferred and not preferred people (57.12±15.42 *versus* 58.00±18.95; F=0.00; p=0.955).

Finally, six on eight preferred owners (75.0% considering only dogs who showed a preference) were the ones who managed the dogs almost totally by themselves; the other two walked the dog and shared other activities with the rest of the family. The dog living in a four-member family who did not show a preference was equally managed by all people.

Discussion

The novelty of the current study consisted in considering the dog as part of a family group: therefore, dog bond to all family members, and not just to one of the owners, has been analysed. Considering the small sample size of this pilot study, results have to be discussed cautiously and not to be regarded as conclusive. However, preliminary data suggest that most dogs living in a multi-member family show a stronger bond to a specific member.

It is possible that results are influenced by the routine of owners' exits. This factor, that was not possible to investigate in the current research, may be responsible for a lower excitement shown by the dog at the exit and re-entrance of a person, although strongly linked to him/her. However, the ASST has been shown to challenge enough the dog because it is performed in an unknown environment, and dogs usually are not used to see their owners going away in an unusual and unknown place. An example is represented by the display of whining.

Whining was almost absent in the first and in the last episode, when all people were present, while it can be observed an increase when one of the owners (or two) left the room and a second increase, particularly high, during the complete isolation. The increase of whining can be considered as an indicator of stress caused by separation (Palestrini et al., 2010); its display at the exit of a specific person, although the rest of the family was within the room, is likely to be related to a stronger bond to that person.

It could also be hypothesized that the repetition of the exiting-entering procedure may progressively get the animal used to this event and therefore showing lower signs of separation distress from time to time. This was not observed indeed, as peaks of time spent close to the door were observed also for the last person who exited (e.g. for dogs I and L).

Based on what Bowlby & Ainsworth observed in children (Ainsworth & Bell, 1970; Bowlby, 1988), also in dogs exploration and individual play are behaviors indicative of the secure base effect, being more displayed in the presence of the attachment figure (Mariti et al., 2013) regardless of the order of execution of episodes (Palmer & Custance, 2008). As for exploration, in the current research it was observed that such behavior had a high spike during the first episode, then a rapid decline, but the following trend is very variable according to the examined subject, achieving the minimum when dogs are in complete isolation. A second spike was observed at the re-entering of a specific owner, regardless of the order he/she left the room, suggesting that this person was the attachment figure and that he/she could act as a secure base. The same results were found for individual play. Although the statistical analysis did not provide significant results, such results suggest that most dogs living in a multi-member family use one owner as a secure base.

Results of the current study suggest that 8 out of 11 tested dogs (72.7%) showed a stronger bond towards one of the owners, while 3 of them did not show a preference for a certain member of the family.

In the protocol used for the current research, the stranger was always played by the same person, a woman. This choice was made in order to match previous studies on dog attachment to people, and it is justified by the fact that women appear to be preferred by dogs when approached or touched (Hennessy et al., 1998; Wells & Hepper, 1999). As for interactions of men and women with their own dog, Prato-Previde and colleagues (2006) only found a gender-related difference in the use of verbal communication, more relevant for women, while they do not differ for play and affiliative behaviors. Such lack of difference may explain what observed in the current study for the gender, that did not result a discriminating factor for dogs' preference. Unfortunately, it was not possible to assess a possible preference based on dog sex, due to the small sample and the disparity in the number of tested female and male dogs.

Comparing data obtained for the L.A.P.S. and the results of the behavioral tests, it did not emerge a correlation between the level of attachment of a specific owner to his/her dog and the dog bond towards that specific owner. It may be hypothesized that the level of attachment of a

person to the dog is not necessarily related to the behavior he/she shows to the dog, and consequently dog bond to that person may not be highly affected by that factor *per se*.

Differently from what observed by Topál et al., (1998), it was not observed a reduction in the contact/proximity to door/chair/shoe when increasing the number of family members. From tables 3, 4 and 5, it emerged instead that, as reported by Bowlby in children, in dogs living in three or four-people families who showed a preferential attachment, it was possible to observe a kind of hierarchy in the level of attachment towards different owners, with high scores corresponding to one or two owners and low scores corresponding to the others. This does not imply that dogs living in numerous families do not show a lower attachment, as suggested by Topál et al. (1998). It may mean instead that in research on dog attachment to people great attention has to be paid to the person chosen as the attachment figure to be tested. According to the current research, basing the choice on owners' perception may lead to a mistake in 25.0% of cases.

The higher score sometimes obtained by the stranger compared to one or more members of the family can be probably explained by dogs' curiosity and good socialization. The stranger was always played by a woman that, as previously mentioned, belong to the sex dogs prefer to be approached by. As already reported by Palmer & Custance (2008), the majority of tested dogs have approached or sough contact with the stranger since the beginning of the test, as well as when she re-entered the room. Dogs' behavior in this condition is very different from the children's one that, at the stranger entrance, often show behaviors such as turning attention to their mother or going towards them, interpreted as signs of fear of the unknown person (Ainsworth & Bell, 1970). This behavior is normal in well-socialized adult dogs and usually observed in the ASST performed with dogs (Mariti et al., 2014).

A very interesting result concerns the correlation found between the preferred figure of the dog and the role of this person in the management of the dog. In 75.0% of cases the preferred person was the one who almost completely managed the dog; for the remaining two dogs it did not exist such an exclusive relationship, and dogs showed to prefer the person within the family who dealt with walking and food or walking and play. Therefore, for all dogs who showed a preference, being walked by a person appeared as a predisposing factor for establishing a stronger bond. Walking resulted more important than food, play and education, maybe for the benefits dogs gain from it: exercise, social interaction with people and dogs, and environmental stimulation. It is common opinion that maintaining dogs always leashed could diminish such benefits (Bekoff & Meaney, 1997), and in general the increase of shared activities, especially walking, is at the top of advice provided by behaviorists where the relationship needs to be strengthened. As a matter of fact, it has been demonstrated that the dog-owner relationship is more affected by the quality rather than the amount of time spent together (O'Farrell, 1992; Rooney & Bradshaw, 2003; Scott & Fuller, 1958), and the act of feeding is a minor part of the relationship, that does not produce a strong emotional response out of feeding time (Scott & Fuller, 1958).

Although food probably plays an important role in creating affection or anyway a positive interest of dogs towards a specific person (that in this case could be simply explained as classic conditioning), establishing an attachment bond seems to be based on different factors, unrelated to the primary drive of feeding. This is what Bowlby (1988) suggested in his theory of attachment, that seems to perfectly fit also dog-human relationship.

Conclusions

Results suggest that most dog living in a multi-member family show a stronger bond to a specific person in that family, namely that involved in managing the dog, especially walking. Further research is needed to assess the possible influence on dog attachment to people of dog sex, age and breed, besides the duration of living within the family.

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Il cane (*Canis familiaris*) come parte della famiglia: uno studio pilota sull'analisi del legame del cane con tutti i proprietari

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Sintesi

Lo scopo del presente studio è stato quello di valutare se i cani che vivono in una famiglia con diversi membri mostrino un legame più forte nei confronti di una persona specifica e, in questo caso, quali caratteristiche del proprietario o della relazione portino a tale preferenza.

Sono stati testati 11 cani, usando una versione modificata del Strange Situation Test di Ainsworth in cui tutti i membri della famiglia (5 famiglie di 2 componenti, 2 con 3 membri e 4 con 4 componenti) erano presenti contemporaneamente. È stata misurata la durata di 19 comportamenti sociali e non sociali del cane. I comportamenti sociali sono stati verificati nei confronti di ogni membro della famiglia presente.

Per ottenere informazioni sulla relazione tra le persone ed il cane testato. È stato utilizzato un questionario che includeva la Lexington Attachment to Pets Scale.

L'analisi dei dati ha rilevato che la maggior parte dei cani (n = 8; 72,7%) che vivono in una famiglia con più membri, mostrano un legame più forte nei confronti di un membro particolare. I proprietari sono in grado solitamente (75%) di individuare la persona a cui il cane è maggiormente legato.

Non è stata trovata alcuna correlazione tra il livello di attaccamento di uno specifico proprietario ed il legame del cane a quella persona (52,12 ± 15,42 *versus* 58,00 ± 18,95; F = 0,00; p = 0,955).

Tra i cani che hanno mostrato una preferenza, la maggioranza (n = 6; 75,0%) preferiva la persona che si prendeva cura di loro quasi esclusivamente; gli altri due preferivano la persona che in famiglia si occupava delle passeggiate e del cibo o delle passeggiate o del gioco.

Perciò, questi dati sembrano avvalorare l'ipotesi che portare il cane a compiere la passeggiata quotidiana aumenti la possibilità di stabilire un forte legame con l'animale.

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A case of aggressive behavior in a mongrel dog

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Abstract: A male mongrel dog, two years old, was evaluated for aggression and reactivity to environmental stimuli. The dog was a stray dog; in March 2016 the current owner decided to adopt him. In May 2016 the owners decided to contact a veterinary behaviorist for instructions on how to introduce the dog into the new home, with another dog and to control the high level of his reactivity. The animal, in fact, often barked to dogs, people and cars.

A diagnosis of predatory aggression, territorial intraspecific aggression towards unfamiliar dogs, situational anxiety disorder was made. It was recognized as the basis of aggressive behaviors, a component of stress and anxiety, worsened by previous experiences. A significant impulsivity of the dog was also identified which worsened the prognosis.

Fluvoxamine (1.5 mg / kg bid) was prescribed, in view of the new home moving. The owners were advised to start, with a dog trainer, a behavioral modification program aimed to: implementing the relationship, improving communication and reading the dog's signals, reducing conflicts and potentially critical situations. At the first follow up the owner reported that, although there was an improvement in the intensity of the symptoms, the dog appeared slightly worse in interspecific relationships: he had bitten both owners. At the second follow up the interspecific aggressiveness was decidedly worse, and two episodes occurred again. In general, the dog appeared to be much more intolerant to physical contact with the owners. It was decided to modify the pharmacological prescription, replacing Fluvoxamine with Fluoxetine (1 mg/kg sid).

At the third follow up, the dog was decidedly improved. The owners referred the dog was able to rest better during the night and daytime hours, to relax more at home. A significative improvement in the interactions with the owners was referred too. It was therefore decided to gradually wean from the drug. The owners decided to reduce the meetings with the dog trainer, until their complete interruption. At the last follow up, the improvements in inter and intra-specific relations were relevant.

The owners understood that, despite the behavioral modification process and the pharmacological treatment, the dog presents behavioral problems that must be managed carefully, respecting his ethological needs.

Key Words: predatory aggression, intraspecific aggression, dog, anxiety.

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Presentation

A male mongrel dog, two years old, was evaluated for aggression and reactivity to environmental stimuli.

History and presenting signs

The dog was a stray dog; since February 2016 the animal voluntarily begun to follow the current owner with her dog (a neutered male dog, about 4 years old), during their walks in the mountains and in the shops of the town. In March 2016 the current owner decided to adopt the

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dog but, not having the possibility of hosting him at home immediately (she planned to move to a larger house, with garden, in the following months), she was forced to shelter him in fenced land of his property. The dog did not show separation problems and he tolerated well the confinement, without aggressive behavior against foreign person accessing the land.

During this period, the dog was habituated to wear the harness and the muzzle and to be led on the leash. Moreover, the dog was engaged in research and problem-solving games, with excellent results.

When the microchip was applied, a blood test was performed to evaluate renal and hepatic functionality that resulted in the physiological range.

During the veterinary examination the dog growled while was manipulated without attacking. The clinical examination was normal.

The dog made regular walks, lasting 1, 2 hours in the mountains, with the other dog.

In May 2016 the owner decided to contact a veterinary behaviorist for instructions on how to introduce the dog into the new home and to control the high level of reactivity of the dog. The animal, in fact, often barked to dogs and people passing in front of the ground (unfortunately it was not possible to obscure the fence) and cars.

There were also some episodes of aggression towards other male dogs with bad consequences for the animal because the big size of the other dogs.

At the time of the first consultation, the owners have already begun to take the dog into the new home, for progressively longer periods of time. The relationship in the house with the other dog was quite good, because the owner prevented any disputes, principally about the food. The only problem reported by the owner is the difficulty of the dog to relax when he was in the garden. He frequently barked at noises produced by heavy vehicles, at children's voices and distant barking of other dogs.

At home the owners complained that dog tended to jump on tables / furniture / kitchen; they tried to invite him to go away with titbit and/or confining him in the study room in which he often chose to go spontaneously.

Examination

The behavioral consultation was held in a restricted area, with few surrounding stimuli, with both the owners and the other dog. Both dogs were unleashed.

The predatory behavior it was very exacerbated. The dog seemed to use it as a replacement activity. In general, the owners reported a marked reactivity towards cats, cars (apparently not with scooters/ motorcycles/bicycles) and, during walks in the mountains, towards horses and probably wild animals.

Even during the consultation, the dog spent most of the time chasing lizards and he seemed to almost fix on this activity. He barked to the few dogs that passed during the consultation.

The owners decided to take him on excursion with a 10-meter long leash, because sometimes he reached them in the car one hour after the end of the walk. With the long leash, the dog was apparently more relaxed during mountain walks; the owners were able to contain his attempts to chase potential prey.

Difficulties in managing interactions with other dogs, of the same or opposite sex, on a leash or loose were reported.

Regarding the interspecific relationships, the dog did not seem interested in interacting with strangers, and even the interactions with the owners had to be short. According to the owner, the dog seemed to really relax, only when he was pet by a person. During the visit, in the pauses from the activity of patrolling the land and hunting the lizards, the dog returned spontaneously near the owner, sat down, let her caress him for a short while, then he returned to walk away.

- Reproductive behavior: not present. The owners had also decided to neuter the dog.
- Somesthesic behavior: it appeared in the norm; the dog did not seem to devote himself for a long time to self-care.

Diagnosis

Predatory aggression, territorial intraspecific aggression towards unfamiliar dogs, situational anxiety disorder (Overall, 2013). It was recognized as the basis of aggressive behaviors, a component of stress and anxiety, worsened by previous experiences (Landsberg, 2013). A significant impulsivity of the dog which worsened the prognosis was also identified (Overall, 2013; Arata et al., 2014).

Treatment

Fluvoxamine (1.5 mg / kg bid) was prescribed, in view of the new home moving. Fluvoxamine is part of the Selective Serotonin Reuptake Inhibitor (SSRI) drugs, derivatives of Tricyclic Antidepressants, with lower side effects (Fitzegerald et al., 2013) and currently used in the treatment of anxiety related disorders. Fluvoxamine, specifically, is reported in the literature to be particularly effective as a regulator of behavioral sequence, reducing the consummatory phase of behavior and having an important efficacy on reactivity and aggressive behavior (Dehasse, 1999). Therapy was prescribed for a continuous period of at least 6-8 weeks (Overall, 2013). Studies conducted also in human medicine have demonstrated the correlation between low levels of Serotonin and impulsive aggressiveness (Impulse control aggression, Overall, 2013).

The owners were advised to start, with a dog trainer, a behavioral modification program aimed to: implementing the relationship, improving communication and reading the dog's signals, reducing conflicts and potentially critical situations. The pleasant activities (daily excursions) had to be continued. At the same time, desensitization and counterconditioning of the stimuli to which the dog reacted, was started by the dog trainer. Instructions were given on how to change the environment in which the dog lived, by obscuring the fence that surrounded the garden or leaving a radio turned on when the dog was confined in the studio.

Follow up

First follow up

The consultation took place in the same area where the first meeting was held, at the end of July, about 3 weeks after neutering. The dog had gone to live in the new house, and currently the dogs fed a BARF diet. The dog trainer reported an improvement in the dog's learning abilities, but the difficulties in intraspecific relationships persisted.

The owner reported that, although there was an improvement in the intensity of the symptoms, the dog had bitten both owners (without tissues injuries and with a controlled bite) in two specific contexts: the aggression towards the woman was relative to the application of the muzzle after the surgery of neutering; the man was bitten while he was attempting to put the harness on the dog.

According to the owners, the escalation of aggression seemed to be unstructured: the threat phase was very short, almost non-existent, the bite controlled, but the dog did not take long to return to a state of homeostasis as a result of aggression.

In order to avoid conflicts, the owners were advised to avoid potentially dangerous situations:

the harness had to be put on the dog by the woman; the use of the muzzle was suspended; the interaction with strangers avoided.

Second follow up

In December, there was the second follow up. Both owners and the other dog were present. The interspecific aggressiveness was decidedly worse, and two other bite episodes occurred.

In general, the dog appeared to be much more intolerant of manipulation even by owners than in the past. The animal did not allow to be brushed, dried, or caressed too long, and he used aggressive behavior (growling, biting) to get away from the situations causing discomfort.

The owners also reported: increased reactivity of the dog towards the stimuli already previously known, restlessness even at home, frequent nocturnal awakenings, during which the dog tended to scratch the door of the study or the bookcase present in that room. The dog was confined every night in that room to prevent the other dog from eating his food too. When the owners gave him free access to the rest of the house, the dog continued to scratch the doors, or intensely barked in the garden. The dog occasionally hid pieces of dried meat in the garden, defending them for a long time, especially if he felt threatened by the other dog. Among the cohabiting dogs there were episodes of scuffle, without any harm to either of them. Several times the dog soiled at home at night, urinating against a wall / bookcase / sofa and this was a serious problem for the owners.

It was decided to modify the pharmacological prescription, replacing Fluvoxamine with Fluoxetine (1 mg/kg sid).

Third follow up

At the third follow up in September 2017, the dog was strongly improved. The owners referred the dog was able to rest better during the night and daytime hours, to relax more at home (an additional kennel was added). The interspecific relationships improved and the owner had learned how to recognize dog pacification. Licking the lips, turning the head (Rugaas, 2017; Mariti et al., 2017) were the signals most used by the dog during excessive or too long interactions. The casual encounters with other dogs were much improved.

It was therefore decided to gradually wean from the drug. The owners decided to reduce the meetings with the dog trainer, until their complete interruption.

The next meeting was set after 3 months, during which time the owners were required to note down and report any ascribable deterioration of the behavior.

Conclusion

In December the improvements in inter and intra-specific relations were relevant.

The owners understood that despite the behavioral rehabilitation process and the pharmacological treatment, the dog presents behavioral problems that must be managed carefully, respecting his ethological needs.

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Un caso di aggressività in un cane meticcio

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Sintesi

Un maschio meticcio, di due anni di età, è stato portato in consulenza per aggressività e reattività agli stimoli. Il cane era un randagio che gli attuali proprietari adottarono a marzo 2016. A maggio dello stesso anno i proprietari decisero di contattare un veterinario esperto in comportamento per avere informazioni su come introdurre il cane in una nuova abitazione, con un altro cane, e per controllare il suo alto livello di reattività. L'animale, infatti, spesso abbaiava a cani, persone e macchine.

Fu emessa una diagnosi di aggressività predatoria, intraspecifica e territoriale verso cani sconosciuti e disturbo d'ansia situazionale. Si individua inoltre una importante impulsività del cane che rende difficoltosa la gestione dei comportamenti aggressivi.

È stata prescritta Fluvoxamina cpr da 50 mg, al dosaggio di 1,5 mg/kg bid, anche in vista di un definitivo trasloco nella nuova abitazione.

I proprietari sono indirizzati ad iniziare, con un Istruttore cinofilo, un lavoro volto ad implementare la relazione, migliorare la comunicazione e la lettura dei segnali del cane, ridurre i conflitti e le situazioni potenzialmente critiche, quindi lavorando soprattutto sulla prevenzione dell'esposizione agli stimoli.

Al primo follow up la proprietaria riferiva che, nonostante si riscontri un miglioramento dell'intensità dei sintomi, il cane appariva leggermente peggiorato nelle relazioni interspecifiche: ha morso, infatti, entrambi i proprietari.

Al secondo follow up l'aggressività interspecifica risultava decisamente peggiorata: si sono verificate altre due morsicature. In generale, il cane risultava essere molto più insofferente alle manipolazioni anche da parte di proprietari rispetto al passato.

Si decise quindi di modificare la prescrizione farmacologica, rimpiazzando la Fluvoxamina con la Fluoxetina (1 mg/kg sid).

Al terzo follow up il cane era decisamente migliorato. I proprietari raccontarono che il cane riusciva a riposare meglio durante le ore notturne e diurne, a rilassarsi maggiormente in casa e tollerava meglio le interazioni con i proprietari.

Si decise quindi di svezzare gradualmente il farmaco ed i proprietari decisero di ridurre gli incontri con l'istruttore cinofilo fino a sospenderli del tutto.

All'ultimo follow up, i miglioramenti nelle relazioni intra ed interspecifiche erano rilevanti.

Ai proprietari apparve chiara, dopo il percorso riabilitativo e lo svezzamento dal farmaco, la necessità che le problematiche comportamentali del cane continuino ad essere gestite con grande cautela da parte loro, sempre rispettando le esigenze etologiche dell'animale.

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Use of Cyproterone Acetate in two aggressive dogs

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Abstract: Canine aggressions remain one of the main reasons for euthanasia or abandonment.

The neurophysiology of aggression is still incompletely known, but several agents have been reported to play a role in its mechanism: androgens, cortisol, and serotonin. This explains why selective serotonin reuptake inhibitors are commonly used as primary pharmacological treatment. Most dogs can improve with environment and behavior modifications associated with such medication. However, in severe cases, they may not completely manage aggressive behavior or underlying anxiety, and multiple medications may be required. That's why cyproterone acetate, a combined androgen antagonist and antigonadotropin, has been proposed. This particular progestin has a wide range of mechanisms of action, including interactions with serotonin and γ-aminobutyric acid, which explains why cyproterone acetate isn't acting like a simple testosterone reducer, but can be used in a similar way as antipsychotics.

We present 2 cases of dogs exhibiting severe aggressive behaviors despite their primary treatment. In each case, the addition of cyproterone acetate led to a decrease in number and intensity of social aggressions; and the aggressions resumed after its removal. In addition, selected cases included male and female, neutered or not, which suggests that cyproterone acetate can be efficient on both male and female, even neutered ones.

The main side effect reported is a mild increase in appetite, which could have been more important without the primary medication (e.g. fluoxetine). Further studies including more cases are needed to investigate these preliminary findings.

Key Words: dog; social aggression; cyproterone acetate; anxiety; fluoxetine.

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Introduction

Dogs with aggression-based behavior problems are commonly presented for behavior consultation (Fatjo et al., 2007; Landsberg et al., 1997). Moreover, behavioral problems remain the primary reason why dogs are abandoned and euthanized (Marston et al., 2004; Reisner et al., 1994). The treatment of aggression in dogs relies on the combination of risk assessment and safety environmental modifications, associated to behavioral modification and medication.

However, some aggressive behavior in dog can be the result of primary psychiatric disorder (e.g. dysthymia[1]) or linked to an underlying anxiety state and controlling them can be a real challenge for veterinarians. This is for those clinical situations where conventional biological interventions and behavior modification are not efficient enough, that cyproterone acetate has been proposed, in a similar way as antipsychotics.

Cyproterone acetate is a synthetic progestogen with a wide range of mechanisms of action (Bolea-Alamanac et al., 2011). Firstly, it acts via a double mechanism: on one hand it blocks

[1] *The behaviourist community has not been able to engage in creating a consensus terminology in veterinary behavioral medicine* (Overall, 2005) *Hence, the authors have decided to use the French nomenclature to describe the symptoms observed in the presented cases.*

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androgens peripherical action via a competitive inhibition to the testosterone cytosolic receptor, and on the other hand it blocks GnRH secretion leading to a secondary blockage of FSH, LH and testosterone (Gruber & Huber, 2003). This double mechanism could result in lower circulating testosterone levels than what is observed with chemical castration (Lieberman, 2013).

Moreover, it has been established that cyproterone acetate interacts with the GABA receptor subtype A, which is known to have anticonvulsant and anxiolytic properties (Bolea-Alamanac et al., 2011; Gruber & Huber, 2003). This progestin may also reduce levels of 5-hydroxy indole acetic acid and homo vanillic acid, metabolites of serotonin and dopamine, resulting in an increase in the availability of these monoamines in the central nervous system (Gruber & Huber, 2003). Another potential mode of action is that cyproterone may modulate the enzyme 5-alpha reductase, thereby altering the brain concentration of allopregnanolone, a neurosteroid that at high doses has anxiolytic properties and is synthesized both in neurons and glia (Pluchino et al., 2006). Finally, it has been demonstrated that cyproterone acetate binds to opiate receptors in mice and may have a role in the endorphin system (Gruber & Huber, 2003).

Considering the inconsistent and controversial reported effects of castration (Farhoody et al., 2018; Hopkins et al., 1976; Maarschalkerweerd et al., 1997; Neilson et al., 1997), it is very likely that the effects of cyproterone acetate on behavior are not only mediated by its effects on testosterone, but rather by its complex and wide mechanisms of action, especially on GABA and serotonin. This is also for this reason that cyproterone acetate cannot be compared to Deslorelin, which is a gonadotropin-releasing hormone (GnRH) agonist that seems to act in a similar way to castration (Beata et al., 2016; Junaidi et al., 2007; Trigg et al., 2006). As a matter of fact, GnRH agonists and cyproterone are used together in humans to nullify the flare-up effect (i.e. temporary increase in circulating LH and testosterone when using a GnRH agonist) observed when initiating the treatment (Le Dare et al., 2015).

However, there are no published data concerning the use of cyproterone acetate to treat social aggression in dogs, even if Pageat (Pageat, 1998) mentioned it as a possible treatment of hypersensitivity-hyperactivity syndrome and sociopathy in association with carbamazepine.

The objective of these cases report was to acknowledge the interest of adding cyproterone acetate for treatment of severe aggressive behaviors, unresponsive to primary medication.

All the aggression and diagnosis listed in this paper come from the French nomenclature of Veterinary Psychiatry initiated by Pageat in 1998 (Pageat, 1998) and developed further in 2003 by Mege and colleagues (Mege et al., 2003).

CASE 1

Presentation

Case 1 is a 5-year-old female German shepherd, weighting 28 kg, presented for multiple aggressions against humans and dogs. Euthanasia is requested by all the family members except the female owner, who is coming to the behavior consultation.

History and presenting signs

The female dog was raised from a working line and was separated from her mother on purpose at 5 weeks of age. She had bitten over 40 times on humans and dogs and the frequency of the aggressions was increasing to reach several episodes per day. Several of these aggressions were severe enough to require surgery on the victims. Every type of aggressive behavior was produced except predation: from the very moment of her adoption at 8 weeks and both towards humans and dogs she exhibited resource holding aggressions around food and toys, accidents during play sessions, without exhibiting submission or deference signals when yelled at. Territorial aggres-

sions appeared with puberty. Overall, the dog's communication signals were so abnormal that she could not establish any normal relationships. Coercive training, including shock-collar, daily physical punishment and aversive communication, led to fear, pain related aggressions (Masson et al., 2018a) and anxiety (Masson et al., 2018b). According to her owner, she never exhibited submission or deference signals when yelled at.

At the time of the consultation, the muzzled dog exhibited increased vigilance, very high level of arousal, and each stimulus triggered aggression attempts.

Physical examination findings and laboratory results

Because of her anxiety and aggressivity level, no exam could be performed without sedation. Blood and urinalysis were in the normal range (including blood count, basic biochemistry, cortisol, fThyroxin and TSH).

Diagnosis

A diagnosis of primary dysocialization was established (Table 1).

Table 1. Diagnosis criteria of primary dysocialization.

The following criteria must be present:
• Aggressions in resource holding situations that are present before puberty (often as early as 3 months old)
• No deference posture can be observed in the dog's communication
• Lack of stop signal
• Lack of bite inhibition
• Intraspecific communication trouble: incapacity to recognize appeasing or deference signals leading to regular aggressions with other dogs

Because brutal aggressions existed from adoption and were not fear-related, they could origin neither from aversive training nor from owners' communication. The dog was hyperactive, but this diagnosis alone does not explain any aggressivity.

Prognosis was guarded considering owner's request, age of the dog, duration of the problem and severity of the diagnosis itself.

Treatment plan

Behavioral treatment: the behavioral part of the treatment plan consisted in safety management measures (Horwitz, 2008), behavioral modification (Herron et al., 2009), training and family strategies (Table 2).

Medication: fluoxetine 4 mg/kg single in day; such dosage is higher than the usual ones described in the literature, but a previous publication reports its use at this dose (Masson & Gaultier, 2018).

Monitoring: number of aggressive-related behaviors per day (snarling, lips lifting, growling, biting) was assessed on the day of consultation and the owner was requested to build an agenda of the upcoming aggressions for the weeks coming.

Table 2. Behavioral part of the treatment plan.

	Before consultation	After consultation
Safety management measures	• Basket muzzle occasionally veterinary visit, some of the walks	• Basket muzzle for all walks • Lock the dog in a garage during all visits
Behavioral modification	• Knowledge of all obedience orders	• Continue to train basic orders, insisting on look • Work on calm protocol
Training	• Physical punishment • Shock collar • Assertive communication • Assertive training	• No physical punishment • No shock collar • Calm communication • Positive training
Family strategies	• Avoiding most of the contacts with outside family members • Attempts to obtain submission when aggressions occur	• Avoiding all contacts outside of the owners • Exclusion of the dog when aggressions occur

Follow up

Results of the treatment plan outcome over the course of time are summarized in Table 3.

Table 3. Evolution of the dog behavior as a function of the treatment plan.

Before consultation and since adoption	Under fluoxetine + behavioral treatment	Under fluoxetine + cyproterone acetate + behavioral treatment
• Brutal and severe aggressions towards humans and dogs	• Reduction of frequency and intensity (50%)	• Reduction of frequency and intensity (90%)
• No submissive or deference signals	• No submissive or deference signals	• Deference signals are observed with the owners
• Impossibility to establish normal relationship with humans or dogs	• Impossibility to establish normal relationship with humans or dogs	• Normal relationships with her owners are possible
• Impulsivity	• Reduction of impulsivity (80%)	• Reduction of impulsivity (90%)
• Lack of bite inhibition	• Lack of bite inhibition	• Lack of bite inhibition

First follow-up was conducted 6 weeks after the initial consultation. The dog improved with this treatment protocol and euthanasia was set aside. However, anxiety was still present, and aggressions were reported at 2 to 5 times a week (snarling and growling but no biting), which was still high considering the extreme caution taken to avoid contacts and triggers. The same treatment plan was continued for 6 months with regular laboratory monitoring (blood count and hepatic parameters) but improvement remained mitigated with several weekly aggressions (no bites). The dog communication improved mildly, but the arousal and the motivation to act in an aggressive manner were still very present.

New medication was added after 6 months of fluoxetine alone: cyproterone acetate 2 mg/kg bis in day.

Aggressive behaviors frequency started to decrease in less than a week. Only 4 aggressive episodes happened during the first month and only 2 were recorded in the next month. The owners reported a heavily decreasing in will to react aggressively and vigilance in the house, as well as an increasing ability to settle down and tolerate petting and interactions in general which improved the relationship with the owners. According to them, she was spontaneously engaging less in game and exploration behaviors, but when stimulated by them to do it, she would respond like before.

An increase in the dog appetite was reported. Blood sample showed no modification. Treatment was adapted to 1 mg/kg bid for cyproterone acetate to avoid possible long-term side effects. For over a year, aggressive behaviors stayed stable at 1 per 2 months, and were limited to snarling, with the dog able to engage in another behavior on queue easily. Again, no blood counts and biochemistry were normal.

Owners decided to stop cyproterone acetate for financial reasons.

Consequently, motivation for food decreased and aggressive behaviors increased, going back to the level of aggressions witnesses before cyproterone acetate. This lasted for 2 years, with a bite resulting in hematoma on the owner's arm during a grooming session. Cyproterone acetate was added again with the same dosage protocol resulting in the same clinical improvements as the first time, despite the fact that she had been neutered in between.

This suggests that cyproterone acetate can be efficient on neutered subjects too. Cyproterone acetate was stopped again after 18 months for financial reasons, with the same outcome.

She ended under fluoxetine alone and was euthanized at the age of 13 years after a severe bite on the owner's arm.

CASE 2
Presentation

Case 2 is a 4-year-old neutered male English Spaniel Cocker, weighting 13 kg. He is presented at the consultation for intense aggressions that appeared around the age of ten months. Owners describe him as a gentle dog 95% of the time that suddenly changes his look and becomes very aggressive without reason.

History and presenting signs

From the adoption at 2 months old to the age of ten months, the dog behaved perfectly normally. Communication and training were of good quality.

At 10 months, the dog's mood changed by crises lasting a few hours, sometimes happening two days in a row and sometimes not happening for several weeks. Between those episodes, the dog was perfectly normal. During the crises the owners could see his pupils dilated and brutal aggressions could then occur: the dog would growl snarl and attempt to bite around specific resources depending on the episode (a friend's dog, a seatbelt, a sport bag). He could also seek petting and suddenly threaten the owner. According to the owners there was no regular pattern or logical explanation to the aggressive events.

For instance, one day the dog was in the car, on the backseat as always and when the male owner moved his hand to grab the seatbelt, the dog jumped on it, shredding it apart. The owner managed to not get bitten. The dog continued to growl, being very agitated and barking at any attempt to approach the car with dilated pupils for over 10 minutes. The owners waited him to calm down, took a few minutes to walk him out of the car, after which the dog acted normal for the rest of the day.

The owners never reacted in an aversive manner to the guarding episodes: they were waiting the mydriasis to stop, and they could tell when they could interact with the dog again. They looked for solutions: neutering was advised by their veterinarian but did not consistently changed the dog behavior or mood changes.

At the time of the consultation, they had been able to habituate their dog to wear a basket muzzle for each car travel, which made their living possible without too many risks.

Physical examination findings and laboratory results

The physical examination was perfectly normal. The dog was brought to the car and out, manipulated for the examination, but no aggressive reaction was observed during the exam. A recent complete blood analysis had already been done by the referring veterinarian without any abnormal findings.

Diagnosis

In this case, the diagnosis criteria were quite caricatural and dysthymia was the most probable diagnosis (Table 4). This mood disorder is often compared to human bipolar disorder.

Table 4. Diagnosis criteria of dysthymia.

One mandatory criterion: brutal and repeated apparition of productive episodes, characterized by an elevation of vigilance level and activity. In addition, 3 symptoms amongst the following must be present:
• Irritation aggression, brutal, severe and without control from the first episode
• Stereotypic behavior
• Fixity phases with mydriasis
• Object guarding
• Search for contact and petting accompanied with threatening
• Hyperphagia
• Hyposomnia

Differential diagnosis included neurological disorder (partial epileptic crisis, brain tumor) and the interest of MRI was discussed with the owners. Communication trouble was ruled out because of the high quality of the relationship between the dog and the owners

Treatment plan

Behavioral treatment: the behavioral part of the treatment plan consisted in safety management measures (Horwitz, 2008), behavioral modification (Herron et al., 2009), training and family strategies (Table 5).

Medication: the first drug prescribed was fluoxetine at 2.3 mg/kg single in day.

Monitoring: the owners were asked to record every mood change, including starting context, duration and ending context.

Table 5. Behavioral part of the treatment plan.

	Before consultation	After consultation
Safety management measures	• Basket muzzle during car rides • Inconsistent isolation of the dog when a crisis started	• Basket muzzle during car rides • Systematic isolation of the dog in a specific room during crises
Behavioral modification	None	• Track for subtle physical changes announcing a crisis • Use postural communication to be able to isolate him without touching him
Training	• Knowledge of all obedience orders using positive training	• Continue practicing of all obedience orders using positive training
Family strategies	• Renouncement to certain activities because of the fear of the dog's reactions	• Inclusion of the dog in all family activities outside of the crises to maintain a good relationship

Follow-up

Results of the treatment plan outcome over the course of time are summarized in Table 6.

Table 6. Evolution of the dog behavior as a function of the treatment plan.

Before consultation and since the age of 10 months	Under fluoxétine + behavioral treatment	Under fluoxétine + cyproterone acetate + behavioral treatment
• Brutal aggressions without control towards humans occurring during "crises"	• Reduction of intensity but not frequency of the crises	• Disappearance of the crises

After 2 months, the treatment seemed to affect only intensity but not frequency of the crises.

Cyproterone acetate was added at the dose of 1.9 mg/kg bis in day for 2 months and fluoxetine was lowered to 1.5 mg/kg single in day. Two months later, no new aggressive episode was reported but a change in the dog behavior was observed: he was apathic and the owners reported that their dog was not really willing to engage in play activities. His affiliative behavior was changed too: he was not willing to interact with owners and was staying still in his basket if no one was stimulating him. His appetite increased slightly with the treatment adjustment. The dosage of cyproterone acetate was divided by two, putting the new dosage at 1 mg/kg bis in day.

The dosage seemed to fit the dog well and the owners reported a will to play close to its initial level before treatment. Affiliative behavior came back to normal as well.

A blood count and chemistry were required every 6 months to monitor a possible elevation in hepatic enzymes. Safety measures were maintained. Decision was made to keep the care this way unless some aggression reappears or blood analysis changes, and until now, two years later, the dog remained stable and no new crisis happened.

Summary box: Danger

Before accepting to treat such dangerous dogs, it is mandatory to establish a contract between the veterinarian and the owner explaining in detail the safety measures to follow. No medical care

should be engaged without such contract. Systematic use of a muzzle when the dog is in contact with people at risk and an isolation zone inside the house are a minimum to require.

Discussion

In dogs, serotonin reuptake inhibitors are used frequently in the pharmacologic treatment of canine aggression (Dodman et al., 1996). In these clinical cases, dogs were given a high dose of fluoxetine (2.3 to 4 mg/kg per day) as first-intent treatment, which is higher than the recommendation of the European Medicines Agency (i.e. 1 to 2 mg/kg per day). The reason for this dosage was the severity of the symptoms, along with the emergency of the situation, which involved possible euthanasia or rehoming if the clinical status of the dogs did not improve quickly (case 1). Until recently, no published study was providing evidence on the safety of fluoxetine at 2-4 mg/kg per day, but the French behaviorist specialists edited a textbook in 2003 (Mege et al., 2003) for the use of fluoxetine at such dosage.

Recently, the use of fluoxetine at a dose of 2-4 mg/kg per day a was presented for the treatment of HSHA syndrome (Masson & Gaultier, 2018). Such treatment is also supported by several studies in humans (Barrickman et al., 1991; Carlisi et al., 2016; Chantiluke et al., 2015) without adverse effects, even on patients with epilepsy (Kanner, 2016).

Those 2 clinical cases illustrate two severe diagnosis that lead to lifelong treatments, where monotherapy with fluoxetine was insufficient to control the symptoms. Carbamazepine (Meyer et al., 2016), which has anti-epileptic and anti-depressant activities, has been reported to control explosive aggression in humans, but can have profound side effects that include agranulocytosis (Avinash et al., 2016).

In humans, cyproterone acetate was used in the treatment of prostate cancer and is now used for androgen-dependent indications in women and precocious puberty in boys. It is also used to control unresponsive aggression, in aggressive dementia, and sex offender treatment (Le Dare et al., 2015). Cyproterone acetate has a weak glucocorticoid activity, which renders its use safer than other progestins; however, several authors report mild adverse effects such as weight gain, loss of libido and gynecomastia, which are due to the lowered serum testosterone concentrations and are mostly reversible (Turner et al., 2013).

For these cases, adding cyproterone acetate was chosen and appeared useful to decrease the dog's arousal and consequently the frequency of the aggressive episodes. In dysthymia, it even acts as an anti-psychotic, able to decrease significantly the number of episodes.

From a phenotypical description of the dog behavior, fluoxetine and cyproterone acetate seem to have complementary roles: fluoxetine is targeting impulsivity and lowers the intensity of the aggression, whereas cyproterone acetate seems to act on arousal and the motivation to perform the aggression. In our two cases, the dogs improved under fluoxetine, exhibiting problematic behaviors less often and with less impulsivity. Nevertheless, their motivation to perform it was nearly intact and it seems that cyproterone acetate was able to act on this part of the behavior.

One of the 2 cases presented resulted in euthanasia. In this case, the dog would have been euthanized at 5 years if she had been unresponsive to treatment. Instead, the behavioral care offered her 8 years, with a good quality of life despite the safety measures taken to make sure that humans other than her owners would be in complete security.

In the presented cases, the main side effect reported was an increased appetite, which is consistent with the cyproterone acetate mode of action (i.e. the inhibition of a testosterone effect). This side effect should be carefully considered before prescribing cyproterone acetate, because it could lead to food-related aggressions. The counterbalance provided by fluoxetine in the presented cases probably prevented such side-effect.

In our study, no dog exhibited any liver enzymes increase. However, hepatotoxicity has been

described and can lead to fatal acute liver failure induced by cyproterone acetate (Bessone et al., 2016; Nour et al., 2017). For this reason, the authors would like to highlight the importance to keep cyproterone acetate as a secondary line treatment, in cases where single fluoxetine therapy does not provide sufficient results, especially when arousal and excessive motivation are still exhibited despite the first medication and only for diagnosis such as primary dysocialization and dysthymia.

Conclusion

The clinical improvement reported in these 2 cases suggest that using cyproterone acetate may be useful for the treatment of severe social aggressive behavior in dogs low responsive to primary fluoxetine medication. The main side effect reported in this study concerned an increase in appetite which was partly balanced by the loss of appetite induced by the primary treatment (e.g. fluoxetine). Further studies including a larger number of dogs are needed to confirm these preliminary findings.

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Conflict of interest

Authors declare no conflict of interest

Authorship statement

The idea of the paper was conceived by Sylvia MASSON and Gerard MULLER
The cases were handled by Sylvia MASSON
The paper was written by Sylvia MASSON and Gerard MULLER
All authors have approved the final article

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Utilizzo del Ciproterone acetato in due cani aggressivi

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Sintesi

L'aggressività canina rimane una delle principali ragioni di eutanasia ed abbandono del cane.

La neurofisiologia dell'aggressività è ancora parzialmente sconosciuta ma diversi agenti rivestono un ruolo importante nel suo meccanismo: androgeni, cortisolo e serotonina.

Questo spiega perché gli inibitori del reuptake della serotonina sono usati comunemente come trattamenti farmacologici primari. La maggior parte dei cani migliora con modificazioni dell'ambiente e tecniche di modificazione comportamentale associate a questi farmaci.

In alcuni casi particolarmente gravi, questi farmaci non sono in grado di gestire perfettamente il comportamento aggressivo o l'ansia sottostante e possono essere necessari altri farmaci.

Questo è il motivo per cui è stato proposto il ciproterone acetato, un composto antagonista degli androgeni e con attività anti-gonadotropinica.

Questo farmaco ha un ampio meccanismo d'azione, includendo le interazioni con la serotonina e l'acido γ-aminobutirrico, che spiega perché il ciproterone acetato non si comporti come un semplice depressore del testosterone ma possa essere utilizzato in maniera simile agli antipsicotici.

Sono presentati due casi di cani che mostrano aggressività grave, nonostante il trattamento farmacologico primario.

In entrambi i casi, la somministrazione di ciproterone acetato ha portato ad una riduzione del numero e dell'intensità delle aggressioni sociali che sono ricomparse dopo la sua sospensione

Inoltre, i casi selezionati includevano maschi e femmine, sterilizzati e non, fatto che suggerisce che il ciproterone acetato possa essere efficace in entrambi i sessi, a prescindere dalla castrazione.

Il principale effetto collaterale riportato è un aumento dell'appetito che può diventare cospicuo, in assenza della fluoxetina.

Ulteriori studi che includano un maggior numero di soggetti sono necessari per approfondire questi risultati.

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Does walking the dog reduce behavioral problems?

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Abstract: In order to investigate the effects of walks on dog behavior, a questionnaire divided into 4 sections was given to dog owners. Based on the 260 questionnaires collected, it was possible to create two groups: one formed by 157 dogs that made daily long walks of a duration greater than one hour (LWD) and another of 103 dogs that took short walks lasting equal to or less than one hour (SWD).

The results obtained show that the performance of a daily walk with the dog of at least one hour has a positive effect on the behavior of the animal, without having to perform sports activities. In fact, dogs that have the possibility to come out for a long walk show a more controlled and polite behavior, as they are less inclined, in a statistically significant way, to pull on a leash, jump on people and chase cats.

It is therefore useful to advise the owners of particularly excitable dogs to take long walks with their animals, if they cannot carry out sporting activities with their animals. This is particularly important for elderly owners who have physical impediments that do not allow them to perform other activities with their dogs.

Key Words: walking the dog, behavioral problems.

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Introduction

Companionship is the most common reason for owing a dog in the Western world (Bennett et al., 2007; Staats et al., 2008).

Dogs can develop a strong attachment bond not only with their conspecific (Mariti et al., 2014; 2017) but also with their owners (Mariti et al., 2013),nevertheless the onset of behavioral problems can sometimes break this relationship. Vacalopoulos & Anderson (1993) reported that up to 90% of dogs may exhibit behaviors that their owners find unacceptable: this fact can become a common cause for dogs to be abandoned and sent to the shelters (Miller et al., 1996; Serpell, 1996; Marston & Bennett, 2003; Shore et al., 2003; Shore, 2005). In fact, over 30% of shelter dogs are abandoned by their owners because of behavior problems (Wells, 2000).

In pet dogs an association between reduced prevalence of undesirable behaviors and attendance at obedience training classes (Clark & Boyer, 1993; Jagoe & Serpell, 1996), engagement with any form of training (Kobelt et al., 2003; Bennett et al., 2007) or high physical activity (Zilocchi et al., 2016) was reported.

Following on from the results of these previous studies, the aim of this research was to evaluate the effect of walking the dog on its behavior.

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Material and methods

In order to investigate the effects of walks on dog behavior, a questionnaire divided into 4 sections was given to dog owners. The first section of the questionnaire regarded the dog (sex, age, reproductive status, origin), the second the owner (sex, age, level of education, profession), the third section concerned the dog management (time spent outside, type of physical activity etc.) and in the final part, 44 multiple-choice questions about dog behavior and their frequency (often, sometimes or never) were asked.

The questionnaires were collected between the months of October and November 2018; all animals were, at the time of the survey, older than one year. Statistical analysis of the data was performedwith the χ^2 test.

Based on the 260 questionnaires collected, it was possible to create two groups: one group was formed by formed by 157 dogs that made daily long walks of a duration greater than one hour (LWD) and the second group consisting of 103 dogs that took short walks lasting equal to or less than one hour (SWD). The statistical analysis did not reveal significant differences as regards the characteristics of thetwo groups which may constitute interfering factors for the research. Table 1 shows characteristics of the subjects in both groups.

Table 1. Characteristics of the dogs belonging to the groups examined.

	LWD N = 157	**SWD N = 103**	**χ^2 test; p**
Dog mean age ± S.D. (months)	54.28 ± 38.47	54.56 ± 40.26	
Male /female dogs (n)	66/87	53/ 50	1.39; 0.238
Male dogs castrated (%)	20.4%	13.6%	1.24; 0.265
Female dogs neutered (%)	35.7%	30.1%	0.63; 0.426
Mongrel dogs (n)	32	29	1.68; 0.195

Results

For brevity, only the results related to excessive agitation and arousal behaviors will be taken into consideration. The percentages of dogs of two groups showing sometimes and often these undesirable behaviors are reported in table 2.

Table 2. Percentages of dogs of two groups showing sometimes and often the undesirable behaviors.

Behavior	**LWD %**	**SWD %**	**χ^2 test; p**
Making too many greetings to the owners when they return	48.6	51.4	4.32; 0.038
Jumping up to the owners (not on their return)	34.4	65.6	9.02; 0.003
Jumping up to another person	33.3	66.7	10.16; 0.001
Escaping from home	0	100	10.29; 0.001
Digging	50.0	50.0	n.s.
Persistently licking other body parts of the owner	59.6	40.4	n.s.
Pulling on a leash	47.5	52.5	4.09; 0.044
Insistently licking him/herself	62.5	37.5	n.s.
Being very agitated and excitable	51.3	48.7	n.s.
Chasing cats	69.2	30.8	3.86; 0.05
Barking at another dogs	68.8	31.3	n.s.

Discussion

Today, many companion dogs occupy a privileged position in our society, living closely with their human caretakers. Sometimes, this intense relationship can be broken if behavioral problems arise, causing the relinquishment of the dog to shelters. These potentially problematic behaviors fall into five factors: disobedience, unfriendliness/aggression, nervousness, anxiety/destructiveness and excitability (Bennet et al., 2007).

The behavior of adult dogs is the result of genetic and epigenetic components. Some studies have shown the effects on the puppy's behavior of the amount of maternal care (Guardini et al., 2015; 2016; 2017) and early manipulation (Gazzano et al., 2008a) as well as the effectiveness of correct dog management advice given to the puppy's owner (Gazzano et al., 2008b).

The data of the present study confirm the results of previous researches showing a significative decrement of behavioral problems in dogs performing physical activity (Zilocchi et al., 2016), attending obedience training classes (Clark & Boyer, 1993; Jagoe & Serpell, 1996) or any form of training (Kobelt et al., 2003; Bennett et al., 2007).

The novelty of the results of this research lies in the demonstration that the performance of a daily walk with the dog of at least one hour has a positive effect on the behavior of the animal, without having to perform sports activities. In fact, dogs that have the possibility to come out for a long walk show a more controlled and polite behavior, as they are less inclined, in a statistically significant way, to pull on a leash, jump on people and chase cats.

It is therefore useful to advise the owners of particularly excitable dogs to take long walks with their animals, if they cannot carry out sporting activities with their animals. This is particularly important for elderly owners who have physical impediments that do not allow them to perform other activities with their dogs.

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Le passeggiate riducono i problemi comportamentali del cane?

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Sintesi

Al fine di chiarire gli effetti delle passeggiate sul comportamento del cane, è stato utilizzato un questionario composto di 4 sezioni. Sulla base dei 260 questionari sono stati creati due gruppi: uno costituito da 157 cani che effettuavano passeggiate giornaliere di lunghezza uguale o superiore ad un'ora (LWD) ed un altro di 103 cani che effettuavano passeggiate di durata inferiore (SWD).

I risultati ottenuti mostrano che effettuare una passeggiata giornaliera di almeno un'ora, con il cane ha un effetto positivo sul comportamento dell'animale, senza che esso debba svolgere un'attività sportiva. Infatti, i cani che hanno la possibilità di uscire per lunghe passeggiate hanno un comportamento più controllato ed educato, poiché sono meno inclini, in modo statisticamente significativo, a tirare al guinzaglio, saltare sopra le persone ed inseguire gatti.

È perciò utile consigliare ai proprietari di cani particolarmente eccitabili di effettuare lunghe passeggiate con i loro animali, qualora non sia possibile far svolgere loro un'attività sportiva. Ciò è particolarmente importante per proprietari anziani che, per impedimenti fisici, non possono svolgere altre attività con il proprio cane.

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Cognitive Dysfunction Syndrome in old dogs

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Abstract: Cognitive Dysfunction Syndrome in old dogs is a pathology from which dogs will more easily suffer, and whose symptoms dogs' owners will have to learn to recognize because dogs' average life is increasing more and more thanks to the considerable improvements in terms of nutrition and of lifestyle. Cognitive Dysfunction Syndrome is a multi-factorial pathology: genetic, environmental and food causes play a determinant role in creating the conditions for the onset of the symptoms; in fact, prevention passes through the improvement of these aspects. Different brain areas are affected and the mechanisms with which the brain tissue is damaged are several. Cognitive Dysfunction Syndrome is characterized by the fact that it is similar to Alzheimer's disease in man, both for what concerns the causes, the pathogenic mechanisms, the affected brain areas and prevention, so much to be used as respective study model.

Key Words: dog, ageing, cognitive dysfunction

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Introduction

Ageing is a process which regards each living species; since it has been studied, ageing has been defined in a lot of different ways: physiological rather than pathological process (Wright et al., 1996); process characterized not only by a loss of functions (senescence), but also by an acquisition of functions (development and growth) (Bowen & Atwood, 2004); process determined by specific genes, which influence the activity of the nervous, endocrine and immune systems (genetic theory) or process mainly influenced by the environment (error theory), to which the random damage of the molecules would add (Weinert & Timiras, 2003).

Ferrara et al. (2005) have summed up the theories that would explain ageing in man: the "redundance" phenomenon, the gene-regulation theory, the theory of evolution, the free-radical theory, the neuroendocrine theory, the theory of the ageing of dismissible bodies, the immune theory.

The dog's ageing could be explained starting from the hypothesis already seen for man, but other factors are involved: size, breed, weight, food as well as lifestyle. Careau et al. (2010) have found a correlation between breed and metabolism, due to which the most "docile" breeds have a longer life expectancy than more "aggressive" breeds: in the first ones metabolism is slower and energy needs are lower. Artificial selection in dogs carried out by man has fostered the appearance of recessive genetic mutations, this is the reason why purebred dogs live less than mixed-breed dogs of the same size (Patronek et al., 1997). The insulin-like growth factor (IGF1) would foster the selection of bigger-sized specimens but with shorter life expectancy because these entities are characterized by a high growth speed, which predisposes to a higher rate of chromosome alterations and, as a result, to a higher probability to develop cancer (Szabò et al., 2016).

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From this quick examination it is possible to infer that ageing must be considered as a multifactorial complex process, in which a lot of mechanisms are relevant and interact (Kowald & Kirkwood, 1996).

Despite the processes determining or influencing it, ageing can be differentiated into: "successful ageing" (absence of pathologies and absence of any minimal functional cognitive loss); "physiological ageing" (absence of pathologies with presence of functional decline); "pathological ageing" (presence of organic diseases and of physical and cognitive disabilities). It is obvious that each species aims at reaching the successful ageing, because in that the organism maintains the ability to respond to environmental stimuli in a proper way managing to live longer and in a healthy way (Waters, 2005).

Anatomical lesions

Cognitive Dysfunction Syndrome in old dogs is caused by the damage to the brain tissue. Different brain areas are affected: the prefrontal cortex, the temporal cortex, the occipital one, the fascia dentata hippocampi are affected by degenerative processes which lead to the death of neurons and reduce neurotransmitter-system function (Siwak-Tapp et al., 2008; Josephs et al., 2011; Mad'Ari et al., 2017). The affected tissues undergo metabolic, neurotropic, structural and vascular modifications. The following modifications occur:

- loss of neurons (due to necrotic and apoptotic processes), together with the reduction of neurogenesis (Head, 2011), with consequent brain atrophy (Youssef et al., 2016);
- accumulation of pathologic proteins (hyperphosphorylated and insoluble tau protein) in cell cytoplasm of neurons (Youssef et al., 2016) and reduced efficiency of "Ubiquitin Proteasome System" of the Hippocampus, which cause the alteration of the normal physiology of the nerve cell, with consequent decrease of cognitive abilities of the old dog (Ghi et al., 2009);
- deposition of β-amyloid plaques in prefrontal, temporal and occipital cortex with consequent cognitive and behavioral changes of the affected dog: appetite, memory, orientation, social interaction, house-soiling are the most involved behavioral aspects (Rofina et al., 2006). The deposition of β-amyloid plaques and the hyperphosphorylation of the tau protein are processes which can co-occur simultaneously during ageing, but they are independent from each other (Pugliese et al., 2006);
- brain-tissue vascular pathologies (deposition of β-amyloid plaques in perivascular area and calcification of capillaries, arterioles and venules), which cause loss of myelin (Colle et al., 2000; Youssef et al., 2016), decrease of blood-brain barrier function and microhaemorrhages (Nichol et al., 2017);
- oxidation processes caused by the production of free radicals inside the neuronal cell, which involve damages of proteins, lipids and of cell nucleotides. A decreased efficiency of antioxidant enzyme systems worsens the cell damage causing nerve demyelination and white-matter damage (Head et al., 2008). A research by Skoumalova et al. (2011), states that, in man, from the interaction between free radicals and cell proteins and lipids Lipofuscin-Like Pigments are generated and that these pigments concentrate also in erythrocytes of people suffering from Alzheimer's disease. This discovery has enabled to use LFPs as biomarkers of Alzheimer's disease. This is not possible in dogs, even if LFPs have been found also in erythrocytes of patients suffering from Cognitive Dysfunction Syndrome and their toxicity for erythrocytes has been proved: it is not sure that these pigments are responsible for the oxidative damage of dog brain tissue (Skoumalova, 2003);
- the old dog's brain tissue can also be affected by inflammatory processes, from whose severity, measured from the astrocytosis level, may depend the intensity of the dog's cognitive deficit (Pugliese et al., 2006);

– neurotransmitter systems can be damaged too: in particular a loss of serotonergic neurons is observed, above all in dogs in which the deposition of β-amyloid plaques in the prefrontal cortex takes place (Bernedo et al., 2009). A loss of noradrenergic neurons has been observed in old dogs suffering from cognitive dysfunction (Insua et al., 2010). Consistent losses of neurons occurs also in the GABAergic and cholinergic systems (Arajuet et al., 2005), these are linked to decreases of attention and memory capacities, responsible, in turn, of the lower ability of old dogs to elaborate new information.

Behavioral signs

All these pathologic processes characterize, in a more or less intense measure, the Cognitive Dysfunction Syndrome in old dogs determining a series of behavioral symptoms, whose severity depends on the intensity of the same processes as well as on the extent of the affected brain areas. It is important to underline that a lot of the symptoms characterizing the Cognitive Dysfunction Syndrome can be caused also by other organ pathologies (Rofina et al., 2001), a pain caused by otitis or a pain of neuro-musculoskeletal origin can trigger aggressive responses as a reaction to a stroke; an important decrease of the auditory and visual functionalities can alter the dogs' usual perception of their environment and thus lead them to show fears, anxieties, aggression and other adjusting strategies, which had never been shown before (Table 1).

Table 1. Behavioral issues linkable with medical problems according Landsberg et al. (2017).

System	Possible causes	Possible behavioral signs
Sensory	Cataracts/lenticular sclerosis	Fear/anxiety
	Loss of vision	Disorientation
	Loss of hearing	Decreased response to stimuli
		Reduced learning ability
		Aggression
		Avoidance
		Vocalisation
Pain/musculoskeletal	Degenerative diseases	Avoidance
	Arthritis	Reduced interest in exercise or play Altered response to stimuli; aggression Reduced self-hygiene Increased vocalisation
	Muscular dystrophy	
Cardiovascular	Mitral insufficiency	Disorientation
	Hypertension	Tiredness or reduced interest in play and activity
	Cardiomyopathy	Withdrawal/avoidance
		Irritability
		Vocalisation
		Changes in appetite
		Fear/anxiety

Endocrine	Diabetes mellitus	All signs of cognitive dysfunction
	Insulinoma	House soiling/urine marking
	Diabetes insipidus	Appetite—increased/decreased
	Hypothyroidism	Activity—increased/decreased/apathy
	Hyperthyroidism	Irritability
	Hyperadrenocorticism	Aggression
	Hypoadrenocorticism	Sleep-wake cycle
		Stereotypic—licking
		Restlessness—pacing
		Vocalisation
Digestive	Dental diseases	Reduced appetite
	Hepatic diseases	Aggression/irritability
	Infectious/inflammatory	Avoidance/withdrawal
	Constipation	House soiling
	Nutritional imbalances	Night-time waking
	Pain	Stereotypic—pacing/licking
		Coprophagia
Urinary	Urinary tract infection	House soiling/marking
	Idiopathic cystitis	Withdrawal/avoidance
	Urolithiasis	Pacing
	Urinary incontinence	Sleep-wake changes
	Urinary tract infection	Aggression

In old dogs the behavioral modification can remain even when the medical cause has been solved. This is due to the fact that the dog has learnt that the new behavior (above all the aggressive one) represents a winning social strategy. The medical therapy is thus useful also for the diagnosis of the Cognitive Dysfunction Syndrome: if the behavioral problem disappears, it means it was determined by the medical cause. If it persists, there is the learning of a new behavioral strategy or there may be two problems: a medical one and a behavioral one, which are not interconnected (Landsberg et al., 2013). In light of these considerations, it is of vital importance to submit the old dog with suspect of Cognitive Dysfunction to medical examinations in order to rule out other possible causes not due to ageing.

The behavioral symptoms can be distinguished into two groups. In the first one there are the ones that have a big impact on the dog's health and, as a result, on the dog – owner relationship (they are less frequent, but they are more reported: aggressions, change of the sleep-wake cycle, house soiling). The second group includes minor behavioral modifications and which have a lower impact on the dog – owner relationship (they are reported less even if they are more frequent: decrease of physical and exploration activity, less search of social contacts) (Landsberg et al., 2017).

The behavioral patterns more reported by owners of old dogs suffering from Cognitive Dysfunction Syndrome are:
- spatial disorientation
- hyperaggression
- decrease of physical and exploration activity (Rosado et al., 2012)
- variation of the sleep-wake cycle
- loss of acquired habits (house soiling)
- less interest for social interactions: about this a research by Mongillo et al. (2013) has pointed

out that the decline of the sensory abilities together with the physical and psychic weakness make the old dog less able to adapt to emotional stress. A consequence would also be anxious behavioral modifications such as separation anxiety.

Diagnosis

Once excluded the medical cause of behavioral modifications, the diagnosis is defined by means of specific questionnaires filled in by the owners. Some problems have been pointed out about the questionnaires. One of the problems is that there are different versions, with different items, which are classified and evaluated differently (Szabò et al., 2016).

Another problem is about the cooperation given by the owners: they are proner to fill in questionnaires with fewer items, even if the longer ones are more accurate and give more precise results. Owners are also prone not to tell their veterinarian about the problem (Mad'Ari et al., 2015), because they do not notice the behavioral modification, or because they consider it physiological for the dog's age, or because they think there is no therapy. This situation has been confirmed also by a research by Landsberg et al. (2013), according to which on a sample of 255 owners of dogs over 8 years old, only 17% of those who recognized at least one behavioral symptom has informed their veterinarian. The problem gets worse if you think that only a minor part of those who refer the symptoms to their veterinarian accept to see a behavioral veterinarian (Osella et al., 2007): from all this you can say that Cognitive Dysfunction Syndrome cases are most likely underestimated.

The categorization of the behavioral symptoms taken into account is done by using three acronyms:

- DISH (Disorientation, alteration of social Interactions, alteration of the normal Sleep-wake cycles, loss of acquired habits (House soiling; Landsberg et al., 2017);
- DISHA (behavioral alterations of DISH and behavioral patterns related to ACTIVITY, such as alteration of appetite, of personal hygiene, of activities in general (Osella et al., 2007);
- DISHAL (previous alterations and behavior modifications related to states of anxiety, as well as to the reduced cognitive and memory abilities (Landsberg et al., 2012).

After the questionnaire has been filled in by the owner, the practitioner assigns a score to each item according to three scales:

1) ARCAD Scale (Age Related Cognitive and Affective Disorders), developed by Pageat (1998), according to which the behavioral disorders of old dogs are divided into three groups: social-behavior disorders (Hyper aggression of old dogs), cognitive-ability disorders (Confusion Syndrome of old dogs), thymic disorders of old dogs (Depression from involution).
2) EDED Scale (Evaluation of Dogs' Emotional and cognitive Disorders), this takes into account emotional-related disorders of dogs of any age, and not only of old dogs, like the ARCAD scale; the final score allows to tell whether the dog is normal or affected from phobias, anxiety or other emotional disorders (Mills et al., 2013).
3) CADES scale (Canine Dementia Scale), proposed by Mad'Ari et al. (2015), it takes into account 17 items divided into 4 groups. The final evaluations are 4: normal ageing (occasional behavioral modifications), ageing with light cognitive handicap (reduction both of contacts with owners and of daily activity), ageing with moderate cognitive handicap (the dog's night activity and inappropriate elimination reach annoying levels for the owner), ageing with severe cognitive handicap (the behavior worsens so much that the owners do not feel like keeping on living together with their dog).

Therapy

The therapy for the Cognitive Dysfunction Syndrome includes the use of drugs and of nutraceutical products. The first ones include PROPENTOFILLYNE (this is able to improve blood perfusion and to inhibit the formation of microthrombi (Donenberg & Landsberg, 2010)); DIAZEPAM and FLUOXETINE (which are able to reduce the effects of stress and depression (Cory, 2013)); SELEGILINE (thanks to its antioxidant property and to its dopaminergic and catecholaminergic activity (Landsberg et al., 2010)); PHOSPHATIDYL SERINE (with neuroprotective properties, able to improve neurotransmission and synaptic functioning (Osella et al., 2007).

Two nutraceutical products have resulted particularly effective: one containing antioxidant principles and mitochondrial co-factors, able to protect the brain tissue from the action of free radicals (Landsberg et al., 2010). The other one made up of phosphatidyl serine, Ginkgobiloba, pyridoxine and dl-α-tocopherol, in addition to antioxidant substances and mitochondrial co-factors; this product has proven able to increase the old dog's memory and learning ability (Colangeli et al., 2005).

Prevention

Several predisposing factors have been into account; concerning sex, breed, size and neutering opinions are different (Azkona et al., 2009; Katina et al., 2016; Mad'Ari et al., 2017). Opinions are the same, instead, for what concerns food and the environment. Regarding food, it has been discovered that dogs fed on uncontrolled diets have 2.8 times higher probability to develop the Cognitive Dysfunction Syndrome than dogs fed on controlled diets (Katina et al., 2016). Araujo et al. (2005) have shown that supplementation of the diet with antioxidant factors (Vitamins A, E, C, selenium) and with mitochondrial cofactors (lipoic acid, carnitine, Coenzyme Q_{10}) can have beneficial effects on the dog's cognitive abilities in both the short and long term, both by protecting the brain tissue from oxidative damage and by increasing the percentage of free polyunsaturated fats (arachidonic acid) inside the neuronal cell, with consequent improvement of neurotransmission and of synaptic function (Bazinet & Layé, 2014).

Regarding the environment, understood as proper relationship with the owner, regular contacts with other dogs, as well as regular physical and mental exercise (problem solving and sniffing practice), it has been shown how it can postpone the onset of the first symptoms of the Cognitive Dysfunction Syndrome, as well as slow down its course, in case it has already started (Mad'Ari et al., 2017). These results have been confirmed also by a research by Chapagain et al., (2017), which showed that, even if the ability of concentration and of keeping attention decreases with ageing, physical and mental exercises carried out during the dog's whole life prevent the clinical symptoms of cognitive decline.

Conclusions

Dogs live longer and longer thanks to the advances made both in Veterinary Medicine and in studies about animals' nutrition and food.

Of course, this involves a price to pay: AGEING. As any other part of the organism also the nervous system undergoes some modifications as time goes by: it is important that the onset of behavioral and cognitive symptoms typical of the brain malfunction takes place as late as possible and, in case, that the symptoms are as light as possible, in order to ensure a good quality life to old dogs and to their owners. In this regard the knowledge of the risk factors and the

therapeutic and prevention measures of the Cognitive Dysfunction become essential. A very important role will be more and more played by an early diagnosis: biochemical diagnostics is already being studied, which aims at identifying the markers that can be more linked to animals' dementia. The cerebrospinal fluid and blood are the fluids who can better serve this research because the first one contains the higher concentrations of them, while the second one is easier to use. The identifying technique of these markers is represented by the immunologic dosage, thanks to which it is possible to identify the protein elements that typically appear in case of a pathology of the nervous system; unluckily the standard procedures necessary to avoid diagnostic errors have not been validated yet (Kovacek et al., 2017).

Considerable advances have been recorded also in therapy these latest years; the effectiveness of new methods is already being studied, such as vaccination against β-amyloid proteins and the use of stem cells obtained from the olfactory mucosa and implanted in the cisterna magna (Veron et al., 2014).

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Sindrome di disfunzione cognitiva nei cani anziani

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Sintesi

La Sindrome di Disfunzione Cognitiva nei cani anziani è una patologia di cui i cani soffriranno sempre più facilmente e i cui sintomi i proprietari dei cani dovranno imparare a riconoscere perché la vita media dei cani sta aumentando, grazie ai notevoli miglioramenti in termini di nutrizione e di stile di vita. La Sindrome di Disfunzione Cognitiva è una patologia multi fattoriale: le cause genetiche, ambientali e alimentari giocano un ruolo determinante nel creare le condizioni per l'insorgenza dei sintomi; infatti la prevenzione passa attraverso il miglioramento di questi aspetti. Diverse aree del cervello sono interessate e i meccanismi con cui il tessuto cerebrale è danneggiato sono diversi. La Sindrome di Disfunzione Cognitiva è caratterizzata dal fatto che è simile al morbo di Alzheimer nell'uomo, sia per quanto riguarda le cause, i meccanismi patogenetici, le aree cerebrali interessate e la prevenzione, tanto da essere utilizzato come rispettivo modello di studio.