



Ethology

# Relationship between sociability toward humans and physiological stress in dogs

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ABSTRACT. Sociability is an essential trait for dogs to successfully interact with humans. In this study, the relationship between sociability and physiological stress was examined. Additionally, whether differences exist between companion dogs (C group) and shelter dogs (S group) was examined. Overall, healthy 37 dogs (C group=21 and S group=16) were examined. After 5 min of walking, the dog and the owner (or the chief manager) rested freely in the experimental location for 5 min. The behavioral test with 6 categories was conducted to evaluate sociability over 4 min. The establishment of two groups (H group=dogs with high sociability; L group=dogs with low sociability) was supported by the statistical results of the behavioral tests. Saliva was collected before (P1) and after the test period (P2), and salivary cortisol levels were determined and statistically analyzed. The cortisol concentrations at P2 and the differences in concentrations between P1 and P2 (P2–P1) in the groups with high sociability were significantly lower than those in the groups with low sociability. These results may demonstrate that sociable dogs adapt more comfortably to strangers and unfamiliar situations. Meanwhile, there were significant differences in hormonal results between the C and S groups. For this reason, their sociability should be evaluated using behavioral and physiological assessments before re-adoption to ensure their successful adaptation.

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The tendency to be friendly toward strangers has been proposed to be a personality trait in dogs, and several studies have described this trait as sociability [22]. Although this trait is related to social fearfulness and aggressiveness, it may also contribute to positive interactions with unfamiliar individuals [22]. More sociable animals, such as those who pay more attention to a person, may be more persistent in their attempts to communicate and may be more easily trained [14]. A lack of adequate socialization can result in problematic behaviors, such as aggression, excessive excitability, fearfulness, sexual inadequacy or indifference toward owners [2].

Some dogs may show sensitive responses to strangers, unfamiliar dogs or other animals or places, resulting in excessive stress, because their chances of experiencing various social situations and human interactions may be limited during the critical early period of their life [2, 17, 25]. This characteristic defect could also result in the failure of dogs to be adopted from a shelter due to their inability to adapt to a new family and house [21]. Overall, these situations may severely harm the welfare of dogs.

Many behavioral assessment tests have been used to evaluate temperament and personality in dogs [8, 15, 23, 24], and multiple studies have identified behaviors and physiological responses in dogs when meeting strangers [1, 6, 20]. Some studies have evaluated behavioral traits, especially the sociability of shelter dogs, using various methods to verify the successful re-adoption and adaptation of dogs to new families and surroundings [19, 26]. Overall, research has shown that sociability is one of the most important traits to have a successful adaptation.

Although various measures have been used to evaluate physiological stress in dogs and assess animal welfare [4, 10, 18], measuring salivary cortisol levels has been the preferred method, because this simple and non-invasive procedure minimizes additional physiological changes as a result of the measurement itself [3, 11, 12, 16]. Additionally, measuring salivary cortisol levels reflects physiological changes relatively faster than other methods, allowing immediate stress responses to be monitored [9, 27]. For these reasons, the physiological responses of dogs facing strangers were evaluated based on changes in salivary cortisol levels.

In this study, the dogs analyzed were divided into two groups based on results from a behavioral sociability assessment that took place at an unfamiliar location without their owners. Differences in the degree of physiological stress (as inferred from salivary cortisol levels) resulting from interactions with strangers between dogs with high sociability and low sociability were evaluated. The differences in the sociability and physiological stress response of companion dogs and shelter dogs were also evaluated.

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 Table 1. Sociability assessment categories

	Passive phase (P)	Active phase (A)
Contact latency (CL)	Measured from the time the animal enters the room un	til it makes physical contact with the E for the first time (sec)
Time close to E (TC)	<1 m distance (sec)	
Physical contact (PC)	Duration of the physical contact between the E and the	e subject (sec)
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CL, TC and PC were same measurement in passive and active phase.

# MATERIALS AND METHODS

#### **Subjects**

A total of 37 healthy dogs (21 companion dogs=C group; 16 shelter dogs=S group) were included in the study. All of the dogs in the C group were privately owned and were housed indoors. The applications were submitted by owners on voluntary base via SNU VMTH (Veterinary Medical Teaching Hospital in Seoul National University) advertisement and other website. The mean age was  $4.57 \pm 0.62$  years, and the mean weight was  $5.67 \pm 0.95$  kg. Four neutered males, six intact females and 11 neutered females were included. Three Malteses, one Shih Tzu, two Yorkshire terriers, two Pomeranians, one Cocker spaniel, one Jack Russel terrier, two Chihuahuas, one Japanese chin, one Shetland sheepdog, one Border collie and six mixed breeds were used.

Shelter dogs from Seoul animal rehoming center in Seoul Grand Park were included in the study as the S group. All dogs were neutered (nine males and seven females). The mean estimated age was  $2.81 \pm 0.44$  years, and the mean weight was  $3.67 \pm 0.17$  kg. Six Malteses, three Toy Poodles, two Shih Tzus, one Miniature Pincher, one Yorkshire terrier, one Pomeranian, one Spitz and one mixed breed were included. These individuals had been kept in the center for an average of  $2.37 \pm 0.38$  months.

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University.

## Experimental locations

The experiment for the C group was conducted in the rectangular enclosure  $(1.5 \times 3.5 \text{ m})$  surrounded by a wall (height=1 m) in an empty room at Seoul National University. In the experimental area, one chair  $(0.5 \times 0.5 \text{ m})$ , for the owner or experimenter (E), was situated 3 m from the wall with the door and 0.5 m from the other three walls. Small signs placed at 1 m in front of the chair were used to designate the experimental area. The experimental procedure for S group was similar and took place in an empty room at a Seoul animal rehoming center. The arrangement of the chair and the marks were identical. The rooms used were unfamiliar to all of the dogs and were cleaned before the experiment. All experiments were conducted at similar time around the noon.

## Experimental procedures

Sociability was identified using behavioral assessment tests [1, 14].

## Period 1: with owners (5 min)

The dog was walked for a maximum of 5 min. After walking, the dog and the owner (or the chief manager) were introduced to the testing area and given time to adapt to their new surroundings for 5 min. The owner or the chief manager was allowed to sit in the chair or on the floor for 5 min, hugging the dog or exploring the place only if he or she did not excite the dog. The dogs were denied food and water for at least 1 hr before the test to prevent the saliva from being diluted.

#### Period 2: separation period (6 min)

*Passive phase*: E came into the testing area and collected saliva samples from the dog immediately after the owner or the chief manager left the room (phase 1, P1). Saliva was collected by keeping a SalivaBio infant swab (Salimetrics, Carlsbad, CA, U.S.A.) in the dog's mouth for 1 min. Restraint during sampling without making friendly visual contact, physical contact or name calling was kept to a minimum. After the first sampling, E sat in the chair for 2 min without moving and made visual contact with the dog. When the dog initiated physical contact with E in any way, E petted the dog's head or chin once.

Active phase: After the 2-min passive phase, E stood while making eye contact with the dog and calling him or her by name in a friendly manner. If the dog initiated physical contact with E in any way, E petted the dog's head or chin once. If the dog did not come close to E, the E repeated the dog's name 3 times at intervals of 10 sec. For the S group, the names of dogs that were called out during the experiment were established during their first visit to the center so that they would be familiar to the dogs. After the 2-min active phase (phase 2, P2), saliva was collected in the same manner described previously.

#### Grouping based on the results of the behavioral tests

A total of 6 categories, three from each phase, were used to evaluate the sociability of the dogs [1, 14]. The overall measures are shown in Table 1 (PCL=contact latency at passive phase; PTC=time close to E at passive phase; PPC=physical contact at passive phase; ACL=contact latency at active phase; ATC=time close to E at active phase; and APC=physical contact at active phase).

## Analysis of salivary cortisol concentration

The swab storage tubes (Salimetrics) were refrigerated and centrifuged at 4,000 rpm for 15 min. The separated saliva samples

	H group (n=17)	L group (n=20)		
C group (n=21)	Companion dogs with high sociability (n=8)	Companion dogs with low sociability (n=13)		
	Age (year): $5.12 \pm 1.26^{a}$	Age (year): $4.23 \pm 0.67^{a}$		
	Weight (kg): $6.95 \pm 1.96^{b}$	Weight (kg): $4.88 \pm 0.96^{b}$		
	Sex: MC (2)/F (2)/FS (4)	Sex: MC (3)/F (4)/FS (6)		
	· One Maltese	· Two Malteses		
	· One Shih Tzu	· One Yorkshire terrier		
	· One Yorkshire terrier	· One Chihuahua		
	· Two Chihuahuas	· Two Pomeranians		
	· One Shetland sheepdog	· One Jack Russel terrier		
	· One Cocker spaniel	· One Japanese chin		
	· One Border collie	· Five mixed breeds		
S group (n=16)	Shelter dogs with high sociability (n=9)	Shelter dogs with low sociability (n=7)		
	Age (year): $2.78 \pm 0.66^{a}$	Age (year): $2.86 \pm 0.59^{a}$		
	Weight (kg): $3.91 \pm 0.23^{b}$	Weight (kg): $3.36 \pm 0.21^{b}$		
	Sex: MC (4)/FS (5)	Sex: MC (5)/FS (2)		
	· Three Malteses	· Three Malteses		
	· One Shih Tzu	· One Shih Tzu		
	· One Yorkshire terrier	· One Pomeranian		
	· Three Toy Poodles	· One Miniature Pinscher		
	· One mixed breed	· One Spitz		

Table 2. Grouping using data from sociability measures (K-means cluster analysis)

H group=dogs with high sociability; L group=dogs with low sociability; C group=companion dogs; S group=shelter dogs; MC=male castrated; F=intact female; FS=female spayed. a, b) *P*>0.025.

were stored within 1 hr at  $-70^{\circ}$ C until they were analyzed. Stored, frozen saliva samples were completely thawed at room temperature (10 min) and centrifuged at 4,000 rpm for 15 min. The absorbance of the supernatants from each sample was measured using an expanded-range high-sensitivity salivary cortisol EIA kit (Salimetrics), and salivary cortisol concentrations were calculated as  $\mu g/dl$ . The optical density of the plates was read at 450 nm using a SUNRISE<sup>TM</sup> version 3.31 microplate reader (TECAN, Männedorf, Switzerland).

## Statistical analysis

The results of the behavioral scoring in the sociability assessments and the calculated salivary cortisol levels were analyzed using SPSS software version 21 (SPSS, Chicago, IL, U.S.A.).

The establishment of two groups (H group=dogs with high sociability; L group=dogs with low sociability) was supported by a K-means cluster analysis of the data from the 6 categories of the behavioral tests. Behavioral results of the groups were analyzed by ANOVA. Two-way repeated measured ANOVA was used to assess the hormonal variation across the period. The differences in the concentration between P1 and P2 (P2–P1) of the groups were analyzed by ANOVA. Because we compared between H and L groups, and between C and S groups, significance level of 0.025 (2-tailed) was adopted throughout the analysis with Bonferroni correction.

# RESULTS

## Subjects analysis

All dogs included in the study were classified according to their status and results of sociability behavioral test (Table 2). There were no significant differences in body weight or age among the groups (P>0.025).

## Sociability test results

The results from the 6 categories of behavioral tests from the groups are shown in Table 3. There were significant differences between the H group and the L group for each category (PCL: P < 0.001, PTC: P < 0.001, PPC: P < 0.001, ACL: P < 0.001, ATC: P < 0.001 and APC: P < 0.001). However, the C group and the S group were not significantly different in any category (P > 0.025).

## Salivary cortisol analysis

Age, weight, sex and breed type of the subjects included in this study were not found to have a significant effect on hormonal results (P>0.05).

In the H group, the salivary cortisol concentration at P1 was  $0.3848 \pm 0.0969 \ \mu g/dl$  and at P2 was  $0.3577 \pm 0.0981 \ \mu g/dl$ . In the L group, the concentration at P1 was  $0.5593 \pm 0.0755 \ \mu g/dl$ , and the concentration at P2 was  $0.6527 \pm 0.0781 \ \mu g/dl$ . There were no significant differences between the groups across the period (*P*=0.058). The differences in the concentration between P1 and

	Passive phase (120 sec)			Active phase (120 sec)			
	CL	TC	PC	CL	TC	PC	
H group	$13.18\pm4.80^{\text{ a})}$	$91.24 \pm 5.57^{a)}$	$19.18\pm4.43^{\text{ a})}$	$13.71\pm4.90^{\text{ a})}$	$97.82\pm6.73^{a)}$	$21.29\pm6.07^{\text{ a})}$	
L group	$107.90 \pm 7.64^{a)}$	$20.15 \pm 7.16^{a)}$	$0.35\pm 0.22^{a)}$	$110.00\pm 6.47^{a)}$	$17.80 \pm 8.36^{a)}$	$0.70\pm0.60^{a)}$	
C group	$67.90 \pm 11.98$	$55.76 \pm 10.25$	$9.81\pm3.97$	$79.43\pm10.74$	$52.71\pm11.68$	$9.71 \pm 4.90$	
S group	$59.75\pm14.19$	$48.94 \pm 11.13$	$7.94 \pm 2.87$	$47.81\pm14.45$	$57.00\pm12.96$	$10.75\pm4.07$	

Table 3.	Mean $\pm$ SE	from	sociability	measures	in all	groups	(sec)	)
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CL=Contact latency; TC=Time close to the experimenter; PC=Physical contact; H group=dogs with high sociability; L group=dogs with low sociability; C group=companion dogs; S group=shelter dogs. a) P<0.001.

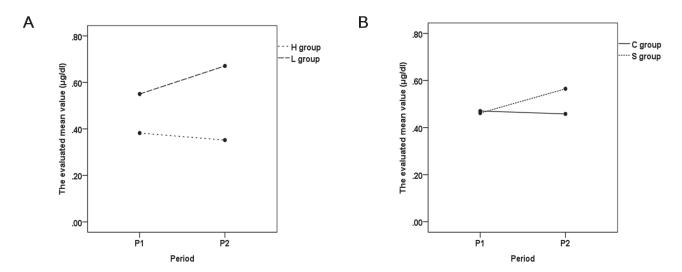


Fig. 1. Variation in salivary cortisol level of the groups. (A) There were no significant differences across the testing period among the groups (*P*=0.058). H group=dogs with high sociability; L group=dogs with low sociability. (B) There were no significant differences across the testing period among the groups (*P*=0.303). C group=companion dogs; S group=shelter dogs.

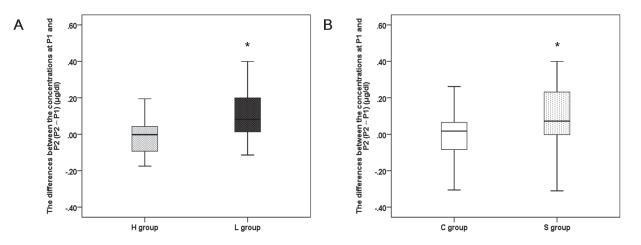


Fig. 2. The differences in concentration between P1 and P2 (P2–P1). (A) There were significant differences between H and L groups (F=10.667, P=0.003). H group=dogs with high sociability; L group=dogs with low sociability. (B) There were significant differences between C and S groups (F=6.224, P=0.018). C group=companion dogs; S group=shelter dogs. \*=significantly different with Bonferroni correction.

P2 (P2–P1) were  $-0.0272 \pm 0.03 \ \mu g/dl$  in the H group and  $0.0933 \pm 0.0371 \ \mu g/dl$  in the L group. These levels were significantly different (F=10.667, *P*=0.003).

In the C group, the concentration at P1 was  $0.5035 \pm 0.0842 \ \mu g/dl$ , and the concentration at P2 was  $0.5037 \pm 0.0823 \ \mu g/dl$ . In the S group, the cortisol concentration at P1 was  $0.4546 \pm 0.0907 \ \mu g/dl$ , and the concentration at P2 was  $0.5439 \pm 0.1082 \ \mu g/dl$ . In the S group, the cortisol concentration at P1 was  $0.4546 \pm 0.0907 \ \mu g/dl$ , and the concentration at P2 was  $0.5439 \pm 0.1082 \ \mu g/dl$ . In there were no significant differences between the groups across the period (*P*=0.303). The differences in the concentration between P1 and P2 (P2–P1) were  $0.0002 \pm 0.0279 \ \mu g/dl$  in the C group and  $0.0893 \pm 0.0458 \ \mu g/dl$  in the S group. These values were significantly different (F=6.224, P=0.018). The overall hormonal results are shown in Figs. 1 and 2.

# DISCUSSION

Sociability measures are important for evaluating the ability of companion dogs to live successfully with humans [24]. The first objective of this study was to confirm the relationship between sociability and physiological stress in dogs. The L group showed significantly high levels of stress variation compared with H group. In other words, dogs with low levels of sociability became more physiologically stressed in response to exposure to strangers and unfamiliar situations. Consequently, less sociable dogs may become extremely stressed in unfamiliar situations, such as abandonment, re-adoption, moving to another location or visiting a veterinarian. If the dog fails to adapt to these various situations and exhibits undesirable behaviors, the possibility of abandonment or abuse increases [21].

The second objective of this study was to compare companion dogs and shelter dogs in terms of their levels of sociability and physiological stress. Some studies have indicated that social isolation and inadequate surroundings may induce various behavioral problems, such as aggressiveness toward strangers or severe timidity, in shelter dogs [1, 8]. In contrast, evaluations of their hormonal changes suggest that dogs in shelters adapt successfully to the shelter environment [13].

The results of the hormonal tests on shelter dogs included in this study were significantly different from those of companion dogs, though the behavioral tests were statistically similar. The results may indicate that when shelter dogs are adopted by new family, the possibility of failure to adapt to the unfamiliar environment may increase; these dogs are increasingly likely to exhibit unacceptable behaviors in some situations due to the high level of physiological stress they experience. For this reason, the sociability of shelter dogs may need to be confirmed using both behavioral and physiological assessments to predict their ability to successfully adapt to a new environment and to reduce the likelihood of a failed re-adoption.

The majority of the dogs showed higher initial cortisol level than the known basal value, which was assessed in their own homes with normal routine and diet [5]. The dogs included in this study were assumed to be experiencing temporary stress, because they had been moved from their familiar surroundings to the unfamiliar experimental area. For this reason, the differences in the concentrations before and after the move (P2–P1) among the groups were also statistically analyzed to account for the increased initial stress level. In addition, there was no significant difference in the concentration at P1 according to age, weight, sex and breed type, consistent with previous studies [5–7].

Meanwhile, the sociability of the dogs may have to be evaluated according to the behavioral and hormonal results of the study, because of the small sample size and the variety of individual differences. It should be noted that the sample size of the study was small and that dogs with a variety of individual histories were included. Therefore, the results of the study may not be representative.

The results of this experiment show that more sociable dogs experience less physiological stress and consequently have a greater ability than less sociable dogs to adapt to various human-based environments. The methods applied in the study may be used to predict dogs' successful adaptation to unfamiliar environments.

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#### REFERENCES

- 1. Barrera, G., Jakovcevic, A., Elgier, A. M., Mustaca, A. and Bentosela, M. 2010. Responses of shelter and pet dogs to an unknown human. J. Vet. Behav. 5: 339–344. [CrossRef]
- 2. Battaglia, C. L. 2009. Periods of early development and the effects of stimulation and social experiences in the canine. J. Vet. Behav. 4: 203–210. [CrossRef]
- Beerda, B., Schilder, M. B., Janssen, N. S. and Mol, J. A. 1996. The use of saliva cortisol, urinary cortisol, and catecholamine measurements for a noninvasive assessment of stress responses in dogs. *Horm. Behav.* 30: 272–279. [Medline] [CrossRef]
- van Hoof, J. A. R., de Vries, H. W. and Mol, J. A. 2000. Behavioural and hormonal indicators of enduring environmental stress in dogs. *Anim. Welf.* 9: 49–62.
- Bennett, A. and Hayssen, V. 2010. Measuring cortisol in hair and saliva from dogs: coat color and pigment differences. *Domest. Anim. Endocrinol.* 39: 171–180. [Medline] [CrossRef]
- Bergamasco, L., Osella, M. C., Savarino, P., Larosa, G., Ozella, L., Manassero, M., Badino, P., Odore, R., Barbero, R. and Re, G. 2010. Heart rate variability and saliva cortisol assessment in shelter dog: Human–animal interaction effects. *Appl. Anim. Behav. Sci.* 125: 56–68. [CrossRef]
- Coppola, C. L., Grandin, T. and Enns, R. M. 2006. Human interaction and cortisol: can human contact reduce stress for shelter dogs? *Physiol. Behav.* 87: 537–541. [Medline] [CrossRef]
- Diederich, C. and Giffroy, J. M. 2006. Behavioural testing in dogs: A review of methodology in search for standardisation. *Appl. Anim. Behav. Sci.* 97: 51–72. [CrossRef]
- 9. Dreschel, N. A. and Granger, D. A. 2009. Methods of collection for salivary cortisol measurement in dogs. *Horm. Behav.* 55: 163–168. [Medline] [CrossRef]
- Haverbeke, A., Diederich, C., Depiereux, E. and Giffroy, J. M. 2008. Cortisol and behavioral responses of working dogs to environmental challenges. *Physiol. Behav.* 93: 59–67. [Medline] [CrossRef]
- 11. Hekman, J. P., Karas, A. Z. and Dreschel, N. A. 2012. Salivary cortisol concentrations and behavior in a population of healthy dogs hospitalized for elective procedures. *Appl. Anim. Behav. Sci.* **141**: 149–157. [Medline] [CrossRef]

- 12. Hellhammer, D. H., Wüst, S. and Kudielka, B. M. 2009. Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology* **34**: 163–171. [Medline] [CrossRef]
- Hiby, E. F., Rooney, N. J. and Bradshaw, J. W. 2006. Behavioural and physiological responses of dogs entering re-homing kennels. *Physiol. Behav.* 89: 385–391. [Medline] [CrossRef]
- 14. Jakovcevic, A., Mustaca, A. and Bentosela, M. 2012. Do more sociable dogs gaze longer to the human face than less sociable ones? *Behav. Processes* **90**: 217–222. [Medline] [CrossRef]
- Jones, A. C. and Gosling, S. D. 2005. Temperament and personality in dogs (*Canis familiaris*): A review and evaluation of past research. *Appl. Anim. Behav. Sci.* 95: 1–53. [CrossRef]
- 16. Kobelt, A. J., Hemsworth, P. H., Barnett, J. L. and Butler, K. L. 2003. Sources of sampling variation in saliva cortisol in dogs. *Res. Vet. Sci.* 75: 157–161. [Medline] [CrossRef]
- 17. Landsberg, G. M., Hunthausen, W. L. and Ackerman, L. J. Developmental, social, and communicative behavior. 2013. pp. 13–16. *In*: Behavior Problems of the Dog and Cat, 3th ed., Saunders Elsevier, St. Louis.
- 18. Möstl, E. and Palme, R. 2002. Hormones as indicators of stress. Domest. Anim. Endocrinol. 23: 67-74. [Medline] [CrossRef]
- 19. Palma, C. D., Viggiano, E., Barillari, E., Palme, R., Dufour, A. B., Fantini, C. and Natoli, E. 2005. Evaluating the temperament in shelter dogs. *Behaviour* 142: 1307–1328. [CrossRef]
- 20. Rehn, T., Handlin, L., Uvnäs-Moberg, K. and Keeling, L. J. 2013. Dogs' endocrine and behavioural responses at reunion are affected by how the human initiates contact. *Physiol. Behav.* **124**: 45–53. [Medline] [CrossRef]
- Salman, M. D., Hutchison, J., Ruch-Gallie, R., Kogan, L., New, J. C., Kass, P. H. and Scarlett, J. M. 2000. Behavioral reasons for relinquishment of dogs and cats to 12 shelters. J. Appl. Anim. Welf. Sci. 3: 93–106. [CrossRef]
- 22. Svartberg, K. 2007. Individual differences in behaviour-dog personality. pp. 182–206. In: The Behavioural Biology of Dogs (Jensen, P. ed.), CABI, Oxfordshire.
- 23. Svartberga, K. and Forkman, B. 2002. Personality traits in the domestic dog (Canis familiaris). Appl. Anim. Behav. Sci. 79: 133–155. [CrossRef]
- 24. Taylor, K. D. and Mills, D. S. 2006. The development and assessment of temperament tests for adult companion dogs. J. Vet. Behav. 1: 94–108. [CrossRef]
- 25. Uzunova, K., Radev, V. and Varliakov, I. 2010. Socialization of puppies-a marker of their future behaviour. Trakia J. Sci. 8: 70-73.
- 26. Valsecchi, P., Barnard, S., Stefanini, C. and Normando, S. 2011. Temperament test for re-homed dogs validated through direct behavioral observation in shelter and home environment. *J. Vet. Behav.* **6**: 161–177. [CrossRef]
- 27. Vincent, I. C. and Michell, A. R. 1992. Comparison of cortisol concentrations in saliva and plasma of dogs. *Res. Vet. Sci.* 53: 342–345. [Medline] [CrossRef]