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Stress in dogs during grooming in a pet shop

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ABSTRACT - The objective was to study the stress level in dogs during grooming services in a pet shop. A total of 55 grooming services carried out in a pet shop in the city of Janaúba, MG, were evaluated and divided into four categories according to sex and body weight. The flowchart of the grooming process contained six steps that began with the transportation of dogs to the pet shop and finished with their return to the place of origin. Behavioral, physiological, and blood component evaluations were performed at different steps of the process. Changes in dog behavior and physiological parameters were observed primarily upon arrival at the pet shop and during drying. Employee characteristics also influenced dogs' behaviors. However, dogs were able to thermoregulate and maintain the homeostasis of leukocytes, glucose and cortisol in the blood.

Keywords: animal behavior, animal welfare, cortisol, pets, physiological parameters

1. Introduction

Hygiene services in pet shops are part of the routine of dog owners because animals are considered family members. Although the grooming process seems to be simple, dogs may be exposed to stressful factors during grooming, such as being handled by strangers, coming into contact with other dogs, being exposed to a wide range of environmental stimuli caused by pet grooming equipment and supplies. The physical environment that includes equipment and furniture of a pet shop and training employees are also important factors in this process. The responses of dogs to grooming are diverse. Some can adapt positively and others become sensitized with the same procedures. When handled improperly, they may suffer trauma or death (Maria et al., 2013).

Stressors can impact dogs hormonally, such as producing changes in cortisol (Medeiros, 2007; Maria, 2015; Oliveira et al., 2016); physiology, such as fluctuations in heart rate, respiratory rate, and rectal temperature (Beerda et al., 1997, 1998; Maria, 2015); and behaviors associated with stress, such as demonstrations of calming signals and postural changes (Beerda et al., 1998; Schilder and van der Borg, 2004; Tod et al., 2005; Rooney et al., 2009; Mariti et al., 2012; Maria, 2015). These changes occur in a way that the animal is able to maintain homeostasis (Beerda et al., 1997); and, because they can be quantified, changes in these values allow us to draw conclusions about the individual's state of stress.

Grooming processes are important repetitive stimuli for pets. Thus, studying how dogs behave during the different steps of grooming becomes necessary to improve management processes in a pet shop and ensure the wellfare of animals during grooming. The objective of this study was to analyze the stress level in dogs during grooming in a pet shop.

2. Material and Methods

This study was approved by the local Ethics Committee on Animal Use and Welfare, case no. 193/2019, and the Ethics Committee for Human Research, case no. 3.479.476/2019.

The study was carried out between May 9 and August 13, 2019, in a pet shop located in Janaúba, MG, Brazil (15°48'13" South latitude, 43°19'3" West longitude, at 510 m above sea level).

A total of 33 castrated dogs (12 males and 21 females) from the Associação de Resgate e Cuidados Animais (ARCA; Animal Rescue and Care Association) were used. All dogs were between two and eight years old. Dogs were previously selected based on health conditions and body weight. They were divided into two body weight categories (animals up to 15 kg and animals over 15 kg) and were not used to the process of grooming in a pet shop.

The grooming area was located at the back of the pet shop, with no physical and visual access for customers with the hygiene procedures performed on the animals. The room was equipped with a grooming bathtub, drying table, waiting cages with iron bars on the outside of the grooming area, bath products, towels, dryers, blowers, and other related objects. There was intense activity from the staff and dogs, including barking, blowing sounds, and ambient music.

Room temperature was measured with a dry bulb thermometer positioned in the center of the cleaning room. The temperature of the bathwater was measured with a food thermometer placed in the plastic container and water samples from all grooming services were collected. The average daily temperature of the experimental period was calculated based on data from the National Institute of Meteorology (INMET). The noises of the grooming area were detected by a smartphone sound level meter application (Tools Dev), and the device was placed at the center of the grooming room.

Stress in dogs was evaluated based on physiological and behavioral responses and quantification of blood components. The flowchart of the grooming process is presented in Figure 1 with the respective evaluations after each step.

The physiological variables analyzed were heart rate (HR), respiratory rate (RR), and rectal temperature (RT). Respiratory rate (breaths min^{-1}) was measured by observing chest movements over 15 seconds. Heart rate (beats min^{-1}) was counted with the aid of a stethoscope placed near the intercostal space for 15 s. Rectal temperature (°C) was measured in the rectum of the animal using a digital thermometer until an audible tone was emitted.

Behavioral responses were observed and noted in a pre-structured ethogram and using videos obtained with three cameras on tripods positioned in front of the animals (Table 1).

Blood samples were taken from 11 randomly selected dogs (six females and five males) by brachial venipuncture using Vacutainer® needles. The blood collected from each animal was split into three tubes: one tube containing an anticoagulant (potassium fluoride), another with ethylenediaminetetraacetic acid (EDTA), and the third tube without anticoagulant. Blood tubes containing potassium fluoride were used to quantify blood glucose by the spectrophotometric procedure using the Bioclin® commercial kit. Blood tubes containing EDTA and those without anticoagulants were sent to an outsourced laboratory for analyzes of leukogram and serum cortisol. Leukogram and serum cortisol were analyzed by automated cell counting (flow cytometry) and chemiluminescence, respectively.

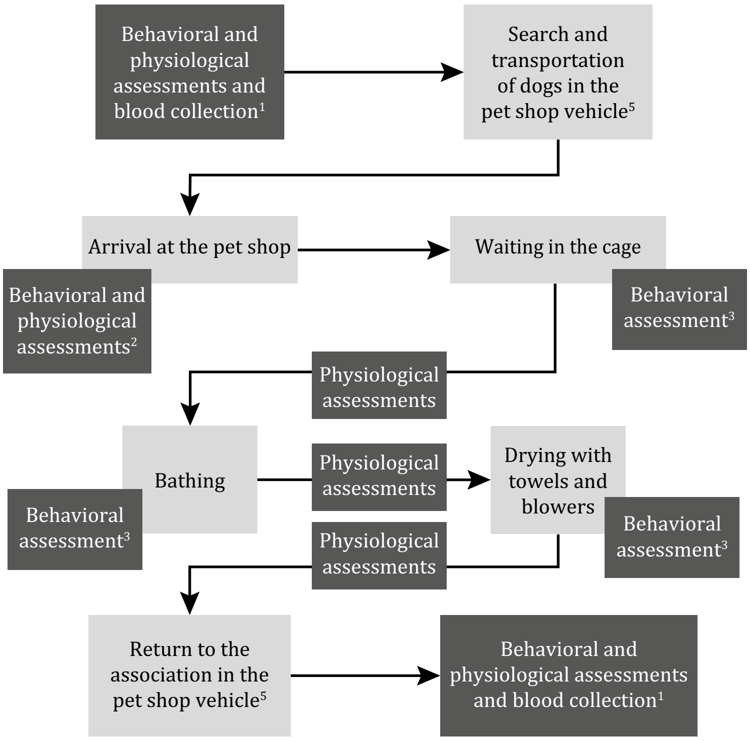
In bathing and drying, the behavior of each employee observed by video analysis during handling performed on the animals was considered. Caresses, conversations, and more rude treatment, such as holding the animal more forcefully or moving it more abruptly were observed. Information on age and professionalization through bath and grooming courses was collected through conversations with the employees, and all data were correlated with the dogs' behavior and physiological responses during bathing and drying, which were the timepoints of greatest contact between dogs and employees.

Data on physiological parameters were analyzed by analysis of variance in a randomized block factorial design with six evaluation timepoints (before transportation to the pet shop, arrival at the pet shop, after waiting in the cage, after bathing, after drying, and after final transportation) and four body weight + sex categories (M≤15: males up to 15 kg; F≤15: females up to 15 kg; M>15: males over 15 kg; F>15: females over 15 kg). Data were compared by Post-Hoc Tukey's test at a significance level of 5% using the Sisvar Statistical Analysis Software version 5.7.

The frequency of behavioral data were compared by the Chi-Square test (P≤0.05) and calculated using the Excel spreadsheet of the Microsoft Office 2013 package.

Hierarchical cluster analysis was performed using the frequencies of behavioral characteristics during grooming steps and dog categories. The original data set for behavioral characteristics was standardized to have zero mean and unit variance. The hierarchical clustering analysis was initially processed because there was no *a priori* information on the number of groups. The Euclidean distance was used as a measure of similarity, and the proximity between groups was determined by the UPGMA approach (unweighted pair group method with arithmetic mean) (Sneath and Sokal, 1973). This analysis was processed using PAST® software.

Dogs' behavioral and physiological parameters and employees' characteristics were correlated using Spearman's coefficient in the statistical software SPSS® version 20.0. The non-parametric Wilcoxon test was used to compare paired results of blood cortisol, glucose levels, and leukogram at P≤0.05 using SPSS®.



[1] Assessments carried out at the association in a place known to dogs.
[2] Evaluation carried out in the waiting room of the pet shop, before allocating the dogs in the cage.
[3] Evaluations made by viewing and later analyzing video footage.
[4] Evaluations carried out right at the end of the flowchart.
[5] Dogs placed in transport boxes and transported in an open body car.

Figure 1 - Flowchart of the grooming process with particular points of physiological and behavioral assessments and blood collections.

Table 1 - Behavior of dogs during grooming in a pet shop

Category	Behavior	Description	Reference
Postural response	Reluctant	Trying to escape the external stimulus	
	Posture low	Head lower than shoulders, tail low, ears lowered	Tod et al. (2005)
	Ears low	Flattened ears	Tod et al. (2005)
	Tail low	The dog holds its tail low or between its legs	Tod et al. (2005)
Calming signals	Raising the paw	Lift a front paw	Beerda et al. (1997, 1998, 1999); Schilder and van der Borg (2004); Rooney et al. (2007); Rugaas (2006); Rooney et al. (2009)
	Licking the nose	Tongue extends upwards to cover nose, before retracting into mouth	Beerda et al. (1997, 1998); Schilder and van der Borg (2004); Tod et al. (2005); Rooney et al. (2007); Rugaas (2006); Rooney et al. (2009)
	Yawning	Mouth open wide for a period of a few seconds, then closes	Beerda et al. (1998); Hennessy et al. (1998); Schilder and van der Borg (2004); Dreschel and Granger (2005); Tod et al. (2005); Rugaas (2006); Rooney et al. (2007)
	Looking away/ turning of the head	Distracts from the stressful stimulus	Rugaas (2006)
	Vocalizing	Barking or whining	Tod et al. (2005)
Physiological behaviors	Salivation	Excessive saliva production	Beerda et al. (1997); Casey (2002); Dreschel and Granger (2005)
	Panting	Rapid open-mouth breathing	Beerda et al. (1997, 1999); Hennessy et al. (1998); Casey (2002); Schilder and van der Borg (2004); Dreschel and Granger (2005); Rooney et al. (2009)
	Shaking	Agitation of the body by involuntary movements in response to fear or cold	Beerda et al. (1999); Dreschel and Granger (2005); Tod et al. (2005); Rooney et al. (2009)
Behaviors caused by handling	Escape attempts/ getting out of the cage	Try to escape while bathing or drying/scratching and pushing against the cage door	
	Falling	Drop completely from the bathtub or drying table	
	Slipping	Slip in the bathtub or drying table	
	Changes of position in the cage	Support of body weight alternates between limbs	

3. Results

The pet shop had an average ambient temperature of 25.8 °C, whereas the temperature of the bathwater averaged 27.7 °C. The mean daily temperature during the experimental period was 24.0 °C. The sounds detected in the grooming room reached, on average, 78.04 dB. Five employees were responsible for the grooming of dogs. Three of them were 16 to 20 years old, and the other two were 21 to 26 years old. Only one employee took a grooming course, whereas others learned from the daily routine of the pet shop.

The results of the physiological variables HR, RR, and RT at different times during the dog hygiene process in a pet shop show that the interaction between dog categories and evaluation timepoints was significant (P≤0.05). There was a significant difference in HR (P≤0.05) according to the timepoints of evaluation. Heart rate was high upon arrival at the pet shop, after bathing, and after drying (Table 2); it was similar within each weight category (P>0.05) regardless of sex. There was no significant difference (P>0.05) in RR between timepoints of evaluation. However, RR of males and females up to 15 kg was different (P≤0.05) within each weight category. Rectal temperature varied after bathing (P≤0.05) in females up to 15 kg. On the other hand, RT was not affected by sex (P>0.05) within each weight category.

In the behavioral assessment, the timepoints in the flowchart showed significant differences for most categories (P≤0.05). Among the evaluated behaviors, postures such as lowered ear, lowered tail, and lowered posture appear more expressive at the time of arrival at the pet shop, during bath, and during drying (Table 3).

Calming signals specific to canine communication such as licking the nose, looking away, and raising the paw were more expressive at the time of arrival at the pet shop, during bath, and during drying (Table 4). In bathing and drying, the reluctance of dogs in the timepoint was greater in most dog categories (Table 3), resulting in the observed slips (Table 4). The presence of vocalization appeared mainly in the bath and drying in the lighter weight dog categories, while waiting in the cage, dogs of all categories were yawning (Table 4).

Escape attempts occurred both while waiting in the cage, in the bath, and during drying, and was more frequent in the latter (Table 5). Escape attempts led some dogs from three of the four categories to fall out of the bathtub during the bath or off the drying table. The change of position was observed in most dog categories when waiting in the cage.

Upon arrival at the pet shop, the percentage of animals that were out of breath was higher compared with the timepoint before leaving the shelter, as well as waiting in the cage and drying (Table 6). Excessive salivation occurred mainly during drying. Most of the physiological variables and behaviors of dogs during bathing and drying did not correlate with the characteristics and behaviors of employees. However, some correlations were moderate and are described as follows: talking to the animal during bathing showed positive correlations with fearful behavior (r = 0.295), vocalizations (r = 0.471), and looking away/turning of the head (r = 0.447). On the other hand, a negative

Table 2 - Means for physiological variables (heart rate, respiratory rate, and rectal temperature) evaluated at different timepoints of dog grooming in a pet shop

	Category	Before trans	After arrival	After waiting	After bathing	After drying	After trans
	M≤15	96.4A	105.2A	105.9A	132.5BC	143.8Cb	109.1AB
Heart rate (beats min^{-1})	F≤15	87.5A	110.6ABC	107.3AB	128.8BC	133.8Cab	117.0BC
	M>15	82.0	98.0	105.5	112.0	114.0a	113.0
CV = 20.8%	F>15	89.1A	106.2AB	101.4AB	123.7B	128.0Cab	85.3AB
	M≤15	32.0	42.5a	47.0	44.7	49.0	41.0
Respiratory rate (breaths min^{-1})	F≤15	40.7	55.3b	49.2	42.6	50.5	38.2
	M>15	45.0	53.0ab	57.0	41.0	42.0	39.0
CV = 49.3%	F>15	43.7	45.0ab	53.2	42.8	52.2	38.6
	M≤15	38.11	38.38	38.66	38.63	38.75	38.58
Rectal temperature (°C)	F≤15	38.14A	38.54AB	38.58AB	38.82B	38.42AB	38.15A
	M>15	38.21	38.47A	38.35	38.87A	38.47	38.27
CV = 1.5%	F>15	38.19	38.58A	38.28	38.85A	38.72	38.21

CV - coefficient of variation.
Evaluation timepoints: before trans - before transportation to the pet shop; after arrival - after arrival at the pet shop; after waiting - after waiting in the cage; after trans - after final transportation.
Categories: M≤15 - males from 0 to 15 kg; F≤15 - females from 0 to 15 kg; M>15 - males over 15 kg; F>15 - females over 15 kg.
Means followed by the same uppercase letters in the row and lowercase letters in the column do not differ by the Tukey's test at a significance level of 5%.

correlation between cuddling dogs during bathing and dogs' falls was observed (r = −0.486). Rude behaviors of the bather correlated with low tail behavior (r = 0.418) and falls (r = 0.367). Negative correlations between the employee's age and behavior of raising the paw (r = −0.440) and panting (r = −0.309) were observed during drying. Employee professional development correlated with heart rate (r = 0.313). Positive correlations were observed between rude employee behaviors and reluctant dogs (r = 0.346), panting (r = 0.354), shaking (r = 0.289), looking away/turning of the head (r = 0.343), and falls (r = 0.403) in dogs.

Grooming steps were separated according to their similarities into four groups (Figure 2A): group 1 (during drying), group 2 (upon arrival at the pet shop and during bathing), group 3 (while waiting in the cage), and group 4 (before transportation and after final transportation). The dog categories males over 15 kg and females over 15 kg showed particular behavior (Figure 2B). Males and females up to 15 kg had similar behavior.

There were no differences (P>0.05) between timepoints of evaluation for all leukocyte components, serum cortisol, and plasma glucose levels in males and females (Table 7). The means for total leukocytes before and after transportation ranged from 6550 to 7684 mm^3 in males and from 7084 to 7048 mm^3 in females, respectively. Only the means for monocytes in males and females before transportation to the pet shop were below the normal.

Table 3 - Relative frequency (%) of dogs that showed postural behavior evaluated in different categories and timepoints of the hygiene process in a pet shop

	Category	Timepoints of evaluation						X^2
		BT	AP	W	B	D	AF	
Reluctance	M≤15	0	0	6.25	25	43.75	12.5	*
	F≤15	0	0	0	17.64	64.7	0	*
	X^2	NS	NS	*	NS	*	*	
	M>15	0	0	0	0	0	0	NS
	F>15	0	0	0	14.28	42.85	0	*
	X^2	NS	NS	NS	*	*	NS	
Posture low	M≤15	0	31.25	6.25	50	75	12.5	*
	F≤15	23.52	41.17	5.88	47.05	76.47	14.28	*
	X^2	*	NS	NS	NS	NS	NS	
	M>15	0	12.5	0	12.5	37.5	0	*
	F>15	7.14	57.14	14.28	35.71	71.42	16.66	*
	X^2	*	*	*	*	*	*	
Low ear	M≤15	0	31.25	6.25	62.5	87.5	12.5	*
	F≤15	17.64	47.05	5.88	64.70	88.23	28.57	*
	X^2	*	NS	NS	NS	NS	*	
	M>15	0	12.5	0	12.5	37.5	0	*
	F>15	0	57.14	14.28	50	78.57	16.66	*
	X^2	*	*	*	*	*	*	
Low tail	M≤15	0	31.25	12.5	43.75	87.5	12.5	*
	F≤15	17.64	35.29	5.88	64.70	70.58	28.57	*
	X^2	*	NS	NS	*	NS	*	
	M>15	0	12.5	12.5	12.5	50	0	*
	F>15	0	42.85	0	42.85	85.71	16.66	*
	X^2	NS	*	*	*	*	*	

NS - not significant.
Evaluation timepoints: BT - before transportation to the pet shop; AP - arrival at the pet shop; W - waiting in the cage; B - during the bath; D - during drying; AF - after final transport.
Categories: M≤15 - males from 0 to 15 kg; F≤15 - females from 0 to 15 kg; M>15 - males over 15 kg; F>15 - females over 15 kg.
Categories and timepoints of evaluation followed by * differ from each other by the Chi-square test at 5% probability.

Table 4 - Relative frequency (%) of dogs that presented calming signals evaluated in different categories and timepoints of the hygiene process in a pet shop

	Category	Timepoints of evaluation						X^2
		BT	AP	W	B	D	AF	
Vocalization	M≤15	0	0	12.5	12.5	12.5	0	*
	F≤15	0	0	17.64	0	11.76	0	*
	X^2	NS	NS	NS	*	NS	NS	
	M>15	0	0	0	0	0	0	NS
	F>15	0	7.14	0	0	0	0	*
	X^2	NS	*	NS	NS	NS	NS	
Lifting the paw	M≤15	0	6.25	0	31.25	43.75	0	*
	F≤15	5.8	0	0	41.17	52.94	0	*
	X^2	NS	NS	NS	NS	NS	NS	
	M>15	0	0	0	0	0	0	NS
	F>15	0	0	0	7.14	28.57	0	*
	X^2	NS	NS	NS	*	*	NS	
Licking the nose	M≤15			75	56.25	75		NS
	F≤15			52.94	94.1	82.3		*
	X^2			*	*	*		
	M>15			87.5	100	75		NS
	F>15			85.71	92.85	92.85		NS
	X^2			*	*	*		
Yawning	M≤15			31.25	0	0		*
	F≤15			11.76	0	5.88		*
	X^2			*	NS	*		
	M>15			12.5	0	0		*
	F>15			35.71	0	14.28		*
	X^2			*	NS	*		
Looking away and/ or turning the head	M≤15			0	25	18.75		*
	F≤15			0	5.88	5.88		NS
	X^2			*	NS	*		
	M>15			0	0	12.5		*
	F>15			0	0	0		NS
	X^2			NS	NS	*		

NS - not significant.
Evaluation timepoints: BT - before transportation to the pet shop; AP - arrival at the pet shop; W - waiting in the cage; B - during the bath; D - during drying; AF - after final transport.
Categories: M≤15 - males from 0 to 15 kg; F≤15 - females from 0 to 15 kg; M>15 - males over 15 kg; F>15 - females over 15 kg.
Categories and timepoints of evaluation followed by * differ from each other by the Chi-square test at 5% probability.
Blank spaces in the table indicate timepoints when the behaviors were not recorded because they need detailed visualization.

4. Discussion

Heart rate varied during grooming events, as described in the flowchart. According to Bodnariu (2008), HR ranges from 100 to 150 bpm in dogs up to 15 kg, and from 60 to 100 bpm in animals over 15 kg. Heart rate was high in dogs of all categories upon arrival at the pet shop, but only in females over 15 kg was it above the normal threshold (Table 2). Respiratory rate, which generally ranges from 10 to 30 breaths min^{-1} in dogs (Bodnariu, 2008), was different between dog categories upon arrival at the pet shop. These changes are associated with the activation of the sympathetic-adrenal-medullary system (SAM) and hypothalamic–pituitary–adrenal (HPA) axes, which are linked to the fight-or-flight response. These responses help to maintain homeostasis during stressful events (Henry, 1992).

High HR and RR values upon arrival at the pet shop (Table 2) showed that the transportation between the departure location (association) and arrival location (pet shop) is a potentially stressful event for dogs. During transportation, animals experience the stress of not knowing where they are (Assis, 2018). Although short, the route from the association to the pet shop has some curves, and the transportation crates were placed on the back of the vehicle. Although the average daily temperature was within the comfort zone for dogs, the exposure of transport boxes to sunlight may partially explain the large number of animals panting upon arrival at the pet shop.

The higher HR and RR after bathing and after drying demonstrate that these grooming steps have an impact on physiological variables. Dogs had direct contact with bathers and dryers during these two steps of the hygiene process. The little familiarity with the bathwater, especially for animals in this study, and noise and pressure of the airflow coming from the blower are stimuli that may have caused changes in these variables.

Rectal temperature averaged 39 °C, which is considered within the normal range. Besides having other causes, changes in RT may be related to acute stress (Maria, 2015); however, in this research, RT did not change between the evaluated timepoints. The use of water at room temperature during grooming and the wind coming from the blower helped to maintain constant RT throughout the events. Maria (2015) compared HR, RT, and RR of dogs before and after bathing and did not find

Table 5 - Relative frequency (%) of dogs that showed behaviors related to handling evaluated in different categories and timepoints of the hygiene process in a pet shop

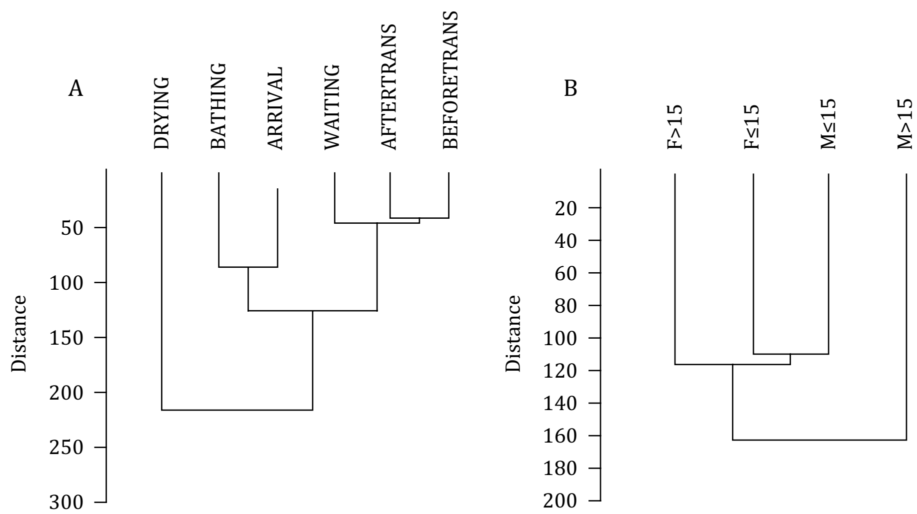
	Category	Timepoints of evaluation			X^2
		W	B	D	
Escape attempts	M≤15	12.5	25	87.5	*
	F≤15	11.76	88.23	94.11	*
	X^2	*	*	*	
	M>15	0	37.5	75	*
	F>15	14.28	57.14	92.85	*
	X^2	*	*	*	
Slipping	M≤15		31.25	18.75	NS
	F≤15		58.82	35.29	*
	X^2		*	*	
	M>15		37.5	12.5	*
	F>15		57.14	21.42	*
	X^2		*	*	
Falling	M≤15		6.25	12.5	NS
	F≤15		0	5.88	*
	X^2		*	*	
	M>15		25	0	*
	F>15		7.14	14.28	NS
	X^2		*	*	
Changing support in the cage	M≤15	75			
	F≤15	58.82			
	X^2	*			
	M>15	75			
	F>15	0			
	X^2	*			

NS - not significant.
Evaluation timepoints: W - waiting in the cage; B - during the bath; D - during drying.
Categories: M≤15 - males from 0 to 15 kg; F≤15 - females from 0 to 15 kg; M>15 - males over 15 kg; F>15 - females over 15 kg.
Categories and timepoints of assessment followed by * differ from each other by the Chi-square test at 5% probability.
Blank spaces in the table indicate timepoints when the behaviors were not recorded because they are not related to it.

Table 6 - Relative frequency (%) of dogs that showed physiological behavior evaluated in different categories and timepoints of the hygiene process in a pet shop

	Category	Timepoints of evaluation						X^2
		BT	AP	W	B	D	AF	
Salivary	M≤15	0	6.25	6.25	6.25	18.75	12.5	*
	F≤15	0	0	0	0	17.64	0	*
	X^2	NS	*	*	*	NS	*	
	M>15	0	0	0	25	12.5	0	*
	F>15	0	0	0	0	14.28	0	*
	X^2	NS	NS	NS	*	NS	NS	
Panting	M≤15	6.25	56.25	56.25	6.25	25.00	0	*
	F≤15	0	11.76	23.52	0	11.76	0	*
	X^2	NS	*	*	NS	NS	NS	
	M>15	0	12.5	37.5	25	0	0	*
	F>15	21.42	21.42	57.14	21.42	35.71	50	*
	X^2	*	NS	*	NS	*	*	
Shaking	M≤15	0	12.5	0	18.75	12.5	0	*
	F≤15	23.52	0	0	5.88	23.52	0	*
	X^2	*	*	NS	*	NS	NS	
	M>15	0	0	0	12.5	0	0	*
	F>15	0	0	0	21.42	0	0	*
	X^2	NS	NS	NS	NS	NS	NS	
Urinating/ Defecating	M≤15	0	6.25	0	0	0	0	*
	F≤15	0	0	0	0	5.88	0	*
	X^2	NS	*	NS	NS	*	NS	
	M>15	0	0	0	0	0	0	NS
	F>15	0	0	0	0	7.14	0	*
	X^2	NS	NS	NS	NS	*	NS	

NS - not significant.
Evaluation timepoints: BT - before transportation to the pet shop; AP - arrival at the pet shop; W - waiting in the cage; B - during the bath; D - during drying; AF - after final transport.
Categories: M≤15 - males from 0 to 15 kg; F≤15 - females from 0 to 15 kg; M>15 - males over 15 kg; F>15 - females over 15 kg.
Categories and timepoints of evaluation followed by * differ from each other by the Chi-square test at 5% probability.



Steps: BEFORETRANS - before transportation to the pet shop; ARRIVAL - arrival at the pet shop; WAITING - waiting in the cage; AFTERTRANS - after final transportation.
Categories: M≤15 - males from 0 to 15 kg; F≤15 - females from 0 to 15 kg; M>15 - males over 15 kg; F>15 - females over 15 kg.

Figure 2 - Dendrograms resulting from hierarchical clustering of grooming steps (A) and categories (B) based on dog behaviors.

Table 7 - Means ± standard deviation and reference values for leukocyte components, serum cortisol, and plasma glucose of 11 dogs at two timepoints during grooming in a pet shop

	Males			Females			Reference range
	Before trans	After trans	P	Before trans	After trans	P	
Leukocytes (mm^3)	6550±2077	7684±2482	0.080	7084±3524	7048±1895	1.000	6000-17000
Neutrophils (mm^3)	4181±1304	4925±938	0.345	4557±2153	4648±1166	0.893	3000-12000
Lymphocytes (mm^3)	1869±1735	1934±1706	0.893	1851±1817	1746±894	0.500	1000-4900
Monocytes (mm^3)	76±97	137±278	1.000	89±94	158±119	0.345	150-1350
Eosinophils (mm^3)	359±429	475±700	0.686	364±345	265±265	0.053	100-1250
Cortisol (mcg dL^{-1})	1.76±1.46	2.06±2.49	0.789	1.81±0.86	1.75±0.71	1.000	0.5-5.5
Glucose (mg dL^{-1})	89.01±9.80	85.43±9.22	0.893	96.19±13.19	98.47±5.46	0.686	73-134*

Evaluation timepoints: before trans - before transportation to the pet shop; after trans - after final transportation.
Means followed by P>0.05 did not differ by the Wilcoxon test for nonparametric paired data.
* Kaneko et al. (1997).
Other reference values were provided along with the results by the Laboratory of Veterinary Analysis Hermes Pardini®.

significant differences between events for HR and RT. However, it is not possible to make a general comparison between our study and that of Maria (2015), since the last evaluated only two grooming steps (before and after bathing), which do not precisely correspond to the moments evaluated in the present study.

The ethological analysis of dogs during grooming showed that some behaviors were more frequent upon arrival at the pet shop. Flattened ears, tail between the legs, and fearful behavior demonstrated the discomfort of animals upon arrival at the pet shop environment. These behaviors are usually observed upon arrival at unknown environments (Beerda et al., 1998) and can trigger physiological changes at that same time. These results corroborate with the findings of Döring et al. (2009), who reported that 60% of dogs brought to a veterinary practice exhibited submissive and fearful behavior. The panting observed in dogs of all categories upon arrival at the pet shop may reflect the thermal stress during transportation.

Behaviors expressed by most categories of dogs while waiting in the cage, in addition to licking the nose, were observed in response to the movement of employees near the cages. According to Firnkes et al. (2017), the approach of a stranger is seen as a threat to dogs. As a result, signs of discomfort increase, such as the behavior of licking the nose. Moreover, the dogs in this study were not used to grooming services and handling in a pet shop and environments with different noise sources. All these stressful factors may have induced some reactions of dogs, which whined and barked sometimes.

Another sign of discomfort is constant yawning when dogs are not sleepy (Rugaas, 1997; Beerda et al., 1998; Hennessy et al., 1998). Waiting in the cage may have increased the exhibition of this behavior in most dog categories because cages restrict the space of dogs and force them to be close to unknown animals. The onset of yawning at this time may also be related to energy expenditure during transport associated with introduction into a new environment, leaving the dogs tired. Thus, they yawned as a result of this process. The animals that arrived at the pet shop breathless continued with this behavior. At this timepoint, it is not possible to separate the effects of environmental temperature from the effects of stress on panting.

Dogs of categories M≤15, F≤15, and F>15 showed more frequent escape behavior. This behavior included attempts to open the door, pressing the front paws against the lock, or even pushing the bars with the nose. Waiting in the cage was a timepoint of physical discomfort, demonstrated by the several changes of position. The space between the wires in the floor grid may have been one of the factors that led the dogs to change position excessively. This behavior, being presented by dogs from different categories, demonstrates that dogs, regardless of their weight or size, are bothered by the cage grid.

The highest frequency of behaviors was exhibited during bathing and drying. Postural responses were more frequent and may be the result of direct contact with bathers and dryers. The reluctance of dogs was evident in response to the discomfort generated at these timepoints. Dogs licked their lips, raised the paws, and looked away, which are indications of acute stress (Beerda et al., 1997).

During drying, the use of the blower is a factor to be considered due to its high noise. Dogs have greater sensitivity and can detect sounds at greater distances than humans (Franzini, 2015). The average noise level in the grooming room was 78.04 dB, which is not harmful to human hearing. However, the act of turning the blower on and off exposes dogs to intermittent noises that resemble the sound phobias many of them exhibit, such as fire and storm noises. This way the signs of stress, such as holding the tail between the legs, shrinking, and shaking are justified by these noises from the sanitation room (Engeland et al., 1990; Beerda et al., 1997; Dreschel and Granger, 2005). Panting and salivation were observed in some dogs during drying. Once the room temperature was within the acceptable range for dogs and their body temperature was maintained, aided by the wet coat, such behaviors are justified by the excessive stress and movement of the dogs when trying to escape the blower wind.

Some dogs slipped in their attempts to escape from bathing and drying. The bathtub was slightly inclined to allow water to drain and the floor was smooth, which contributed to the loss of balance in dogs during handling. Escape attempts were expected because dogs were not familiar with pet shop handling, especially in response to the intense noise coming from the blower and the little familiarity with bathing and water.

Successful escape attempts resulted in falls from the bathtub or drying table. In some cases, the animals were temporarily hanged by the leash attached to the neck collar until the employees were able to put the dogs back to the handling areas. This could have caused severe injuries and demonstrates the discomfort of animals with the handling to which they were subjected. Avoiding accidents such as those in the present study is essential for physical and emotional integrity of animals. The negative correlations between petting the animals and the falls that occurred demonstrate the importance of training employees to deal with adverse situations depending on the characteristics of animals being handled. In the present study, only one employee of the pet shop was trained to work as a dog groomer. Therefore, the staff's lack of preparation for the work became evident.

Despite incidents during bathing and drying, employees had only a small influence on most dog behaviors and physiological responses in these two timepoints. Moreover, only a few significant correlations were moderate. The slight influence of bathers on dogs shows that even talking directly to them, the animals exhibited fearful behavior, vocalized, and looked away, indicating the fear of unknown management. However, the frequency of falls from the bathtub reduced when the bather cuddled the dog. When the employee showed some rude behavior, the dogs displayed the low tail behavior and slipped in the bathtub. Rude behavior of the handler during drying was associated with shaking, looking away behavior, slips, and reluctant behavior of dogs at the time. According to Silva et al. (2019), careful and well-conducted handling without rude reactions contributes to a better outcome and allows appropriate behaviors in animals. therefore, not making grooming a negative experience for animals was a responsibility of the employees, given the fact that animals in this study were not used to handling.

Most of the behaviors indicating discomfort in dogs were reduced after transportation to the association, but submissive behaviors were still observed. The maintenance of these behaviors after returning to the association is due to the dogs' little familiarity with the hygiene procedure. Lore and Eisenberg (1986) reported that females are generally less reactive than males to changes in routine, such as introduction to different handling procedures and new environments. However, females in the present study exhibited a higher frequency of fear-related behaviors such as low ear, tail low, and fearful behavior compared to males. The ethological analysis also showed that, although dogs of the two weight categories exhibited fear-related behaviors, males and females over 15 kg displayed the highest frequency of such behaviors. This difference between the dog categories, in this case, is related to the difficulty of handling heavy animals, which will naturally demand longer grooming times and, consequently, will be exposed to more prolonged stimuli during grooming.

The particularity and similarities between the steps of dog grooming and weight categories (Figure 2) allow us to determine which grooming events and characteristics of dogs deserve attention. Besides producing a loud noise, the blower produces a strong wind-blow on the dogs. Moreover, the influence of rude behavior and of the reluctance the dogs is another crucial aspect of the drying process. The timepoint of waiting in the cage has some particular characteristics, such as location in an outside area without temperature control, the intense movement of employees near the cages, and the close presence of unknown dogs. The cage grid is also another aspect influencing the behavior of dogs.

The arrival in a new environment and the bath were considered similar timepoints (Figure 2A), as they were not used to the environment and the hygiene and practice of bathing. On the other hand, the similarity between the timepoints before and after transportation (Figure 2A) is explained by the fact that the environment where the assessments were carried out is familiar to the dogs. The difference between heavy males and females (Figure 2B) may be related to the difficulty of dealing with heavy dogs associated with sexually dimorphic behaviors such as the fact that females are considered more calm and affectionate and males more active and aggressive (Landsberg et al., 2004). These characteristics influence the way the hygiene management is conducted, reflecting on the dogs' behavioral responses.

Attention must be paid to the equipment, facilities, and proper management according to dog size at each timepoint of the grooming process, since they generate diverse behavioral responses in dogs. However, the overall characteristics of the pet shop of this study could be improved to reduce negative stimuli in dogs. Examples include the use of a vehicle with limited in-car sun exposure, temperature-controlled facilities, use of waiting cages with a smooth surface and without grids, use of blower silencers, use of non-slip surface on the bottom of the bathtub, among others. Moreover, the employees were just doing what was asked of them, and most of them were not qualified for this job. Therefore, they did not understand that some of their attitudes could bring negative consequences to animals.

The analysis of blood samples showed that the timepoints of evaluation did not affect the results of leukogram, serum cortisol, and plasma glucose ($P \leq 0.05$). All these blood components were within the reference values (Table 3). Although dogs faced instances of intense stimuli with a likely increase in the number of leukocytes, this parameter quickly returned to the normal level. The results of the present study corroborate with findings of Oliveira et al. (2016), who assessed the blood count of dogs during grooming in a pet shop in the city of Patos, Paraíba, Brazil, and of Maria (2015), who did not find differences in total leukocyte count of male and female dogs before and after bathing. Under acute stress, the stimuli of a rapid grooming session may trigger changes in the immune system that could lead to leukocytosis. This condition is caused by the action of catecholamines released after stimuli in the sympathetic system (González and Silva, 2003). However, as soon as the stressful stimulus ceases, the leukocytes return to their normal circulating levels (Nelson and Couto, 2005) since catecholamines have a fleeting effect.

Both cortisol and glucose, which is a precursor of energy in timepoints of stress, showed no significant differences between timepoints of evaluation, regardless of the sex category. Thus, the stimuli caused by stressful factors during the grooming process were not able to change serum cortisol levels and elevate blood glucose in dogs. The studies of Medeiros (2007) and Maria (2015) did not find differences in cortisol levels before and after bathing for each sex category. Males and females in the present study were subjected to a similar grooming process, and thus the same stimuli, but no significant differences between steps were observed.

5. Conclusions

Although dogs were not used to the grooming process and exhibited changes in heart rate, respiratory rate, and behavioral parameters, they were able to maintain the homeostasis of leukocytes, cortisol, and blood glucose.

Priority must be given to the welfare of dogs during all stages of the hygiene procedure. Thus, hygiene places need to offer adequate transport service, present a structure of the hygiene environment that is concerned not only with the physical integrity of dogs but also with the training of employees to handle the animals correctly to reduce their sense of fear.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: M. Ferreira. Formal analysis: M. Ferreira, M.A.P. Rodriguez and L.L.S. Oliveira. Investigation: M. Ferreira, L.L.S. Oliveira and M.R. Madureira. Methodology: M. Ferreira, L.L.S. Oliveira, C.M.A. Maranhão and M.V.R. Afonso. Project administration: M.A.P. Rodriguez. Resources: M. Ferreira, M.A.P. Rodriguez, L.L.S. Oliveira, C.M.A. Maranhão, N.J.F. Oliveira and M.R. Madureira. Supervision: M.A.P. Rodriguez. Validation: C.C.S. Carvalho. Visualization: M.A.P. Rodriguez. Writing-original draft: M.A.P. Rodriguez, L.L.S. Oliveira, N.J.F. Oliveira, C.C.S. Carvalho and M.V.R. Afonso. Writing-review & editing: C.C.S. Carvalho.

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