Assessing behavior and stress in two dogs during sessions of a reading-to-a-dog program for children with pervasive developmental disorders

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Abstract: Previous studies suggest an improvement in both reading skills and attitudes towards reading when children read in the presence of a dog.This seems to be related to dogs being fully capable of acting as active and supportive listeners. However, little is known about the potential welfare implications in dogs involved in these activities. Although dogs could receive comfort during a reading session, they might also experience stress, causing a decline in their willingness to work and overall performance. Salivary cortisol and behaviors were analysed in 2 healthy dogs before, during and after 30-minute reading sessions with 4 children with pervasive developmental disorders (PDDs) to identify any signs of stress. Although one dog had significantly high salivary cortisol levels on arrival at the facility, no signs of behavioral or physiological stress were detected in the dogs during and after the sessions. Thus, this particular activity did not negatively affect the welfare of the dogs. Further large-sample studies are needed to more fully explore either the benefits to PDD children or the physiological status of dogs during reading-to-a dog programs, from a "One Health-One Welfare" perspective.

Key Words: stress in dogs; PDDs in children; reading-to-a-dog; dog as listener; shelter.

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Introduction

Dogs are able to offer children a unique type of emotional support in the education setting because they are fully capable of being active, supportive listeners, but are unable to verbally criticise or comment upon a child's reading abilities (Pirrone, 2017). So, it is no surprise that over 70% of young children tend to talk to and confide in animals (Oyama et al., 2017). Dogs may help motivate children to read by increasing relaxation and confidence, reducing blood pressure and offering a non-judgmental, safe environment in which to practice reading (Shaw, 2013). Because improving reading motivation improves reading performance, if children are more motivated to read with a dog then their reading abilities and learning experiences will be enhanced (Hall et al., 2016). Although there is anecdotal evidence that children love reading to animals, and animals, in their turn, love having children read to them, there is a general lack of published experimental data.

Poor literacy skills have substantial health and welfare implications for society and for this reason, reading skills have wide-reaching implications (Mancilla-Martinez & Lesaux, 2010) from early childhood (age 4.5). The first high profile program to advocate children reading to dogs was established in 1999 by Intermountain Therapy Animals, who announced Reading Education Assistance Dogs (READ). The READ program is one of the most comprehensive programs that involve animals to strengthen reading skills (Hall et al., 2016). Only recently, some educational settings have been developed and studied also focusing on PDD (Pervasive Developmental Disorder) children (Grigore and Rusu, 2014) the presence of a therapy dog reading in the presence of dogs.

Although a growing body of evidence supports the rewards and benefits of human-animal

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interactions for humans (Pirrone, 2017), only few investigations have focused on the potential welfare implications for dogs during reading session. The welfare of dogs involved in reading sessions is to be questioned, as social interactions are amongst the most potent stressors a dog can endure (von Holst, 1998; Karatsoreos & McEwen, 2011). Social interactions can be unpredictable, requiring the individual to constantly adapt physiologically and behaviorally to maintain homeostasis (Karatsoreos & McEwen, 2011). The unpredictability of children, particularly if they are PDD children, may be even more challenging for dogs to manage. Dogs could experience stress (both acute and chronic) that might affect their willingness to work in an education setting, thus affecting their performance and excluding them from work (Pirrone, 2017). As with other canine-assisted activities, there is a clear need to objectively evaluate the welfare of dogs during this particular work (Palestrini et al., 2017) through the assessment of stress-dependent behavioral and physiological signs (Beerda et al., 1998; Palestrini et al., 2005; Pirrone et al., 2017).

To the best of our knowledge, this is the first study objectively measuring and compare behaviors and physiological reactions (measured by saliva cortisol sampling) in dogs involved in reading sessions with PDD children.

Materials and methods

Participants

Two spayed female rescue mixed-breed dogs were recruited from the local non-profit Organization "Effetto Palla ONLUS", with the aim of enhancing their socialisation and adoption rates (Hatch, 2007). They were 2 and 8 years old (mean ± Standard Error SE, 5.0 ± 3.0) and weighed between 3 and 18 kg (mean ± SE, 10.5 ± 7.5) at the time of the sampling period. To be eligible for participation in the reading program, the dogs were required to be in good clinical health (i.e., free from pain, external and internal parasites, and immunized). They were subjected to regular health screening and behavioral monitoring by a veterinarian with expertise in animal behavior and welfare. The two dogs were chosen given their kindness and cooperation when handled by children, their interest in people and absence of any signs of anxiety, fearfulness, reactivity or aggression.

Study design

Dogs were assessed while involved in weekly group reading sessions with 5 children (mean age 7±0.45 SE years old), all with a PDD diagnosis.

The reading sessions run for 10 consecutive weeks, once per week, over a period of 70 days. Sampling was carried out during 5 subsequent sessions per dog, that is, 10 sessions in total. Each session was approximately 30 minutes in length. The two dogs were handled by a female veterinarian expert in welfare and behavior, who was familiar with them and was always present during the sessions to guarantee the well-being of the pets. A video camera was set up on a tripod and left running continuously. An experimenter switched the camera on just before the session started and switched it off when the session ended. To be less distracting for the dogs and the children, during the sessions, she usually sat on a sofa. Sessions were performed in a 6×5 carpeted room at the facility, where children usually were involved in other activities, in the presence of a physiologist. In more detail, at reading sessions, 1 visiting dog, 1 dog handler/veterinarian, 1 psychologist, and 1 experimenter were always present. The room temperature ranged between 20° and 24° C.

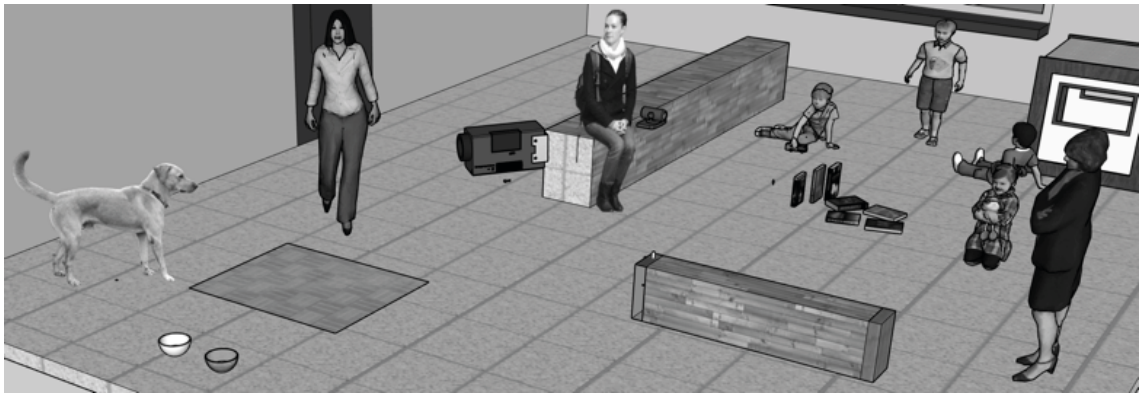

Figure 1. Setting of the room during the reading session.

Two 30-cm-high benches were placed to separate the room into two identical spaces, one for the dog and one for the children, so that direct interaction was discouraged.

No child-initiated contacts with dogs in this study were allowed. Child-animal interaction was limited to verbal contact, where a child talked to the dog to praise her or to ask whether she enjoyed the story or was getting bored. For ethical reasons, dogs were never forced into positions and were able to lie down, drink fresh water, or leave the reading room at any time (Glenk et al., 2014).

Informed consent was obtained from all participants (or their legally authorised representatives), who were previously advised by the facility staff members of an experimenter's presence for the videotaping procedure.

Behavioral assessment

The dog and the handler entered the carpeted room 30 minutes before the beginning of each reading session and left the room 30 minutes after.

The behavior of each dog was videotaped by the experimenter and subsequently analysed before, during and after the reading activity. Animals were off leash at all 3 time points. Analysis of behavior was carried out with focal animal sampling and continuous recording using the Observer XT software package (Noldus Information Technology, 6702 EA Wageningen, The Netherlands). To preserve the anonymity of participants, video recordings were stored in the principal investigator's computer and in the supervisor's computer at the Department of Veterinary Medicine at the University of Milan.

As reported in Table 1, we identified behaviors that could be reliably recognised and defined them on the basis of a literature review (Beerda et al., 1998b; Haverbeke et al., 2008; Pastore et al., 2011; Pirrone et al., 2017).

Behavioral variables were measured in terms of frequency (number of occurrences), relative frequency (the number of occurrences per minute) and/or duration (time spent on a behavior, expressed in seconds) of occurrence during each observation period.

Table 1. List of behaviors and definition used in the study.

Behavior	F/D	Description
Attention-seeking	F	Seeking attention and physical contact from children: nuzzling or pawing for attention, jumping up on, asking to be petted
Avoidance	F	Escape behavior, withdrawal, eyes or head turned away from children
Body shaking	F	Move, shake the body with energy
Changing of posture	F	Frequent changes of position: standing up shortly after sitting/lying down for ≤ 30s
Escaping	F	Try to open the door or to leave the room
Exploring	D	The dog moves slowly, sniffing and investigating the environment
Lips/nose licking	F	Part of the tongue is shown and moves along the upper lip and/or nose
Looking at	F	Looking at children or the handler
Lying down	D	Static position with hindquarters flexed and in contact with the ground; forelimbs are extended but completely in contact with the ground for ≥1
Paw lifting	F	A fore paw is lifted from the ground, flexed into a position of approximately 45°
Persistent self-grooming	F	Oral behavior directed toward dog's own body (licking, chewing skin or coat) for ≥1 min
Recumbent	D	Static position with trunk lying in complete contact with the ground in lateral, sternal, or dorsal recumbency for ≥1 min
Scratching	F	Purposeful movement of limbs to scratch any part of body
Sitting	D	Static position with hindquarters flexed and in contact with the ground; forelimbs are extended with only paws in contact with the ground for ≥ 1 min
Standing	D	Upright static position with at least 3 paws in contact with the ground for ≥1 min
Tail down	F	Lowered position of tail
Vocalising	F	Any form of vocalisation, including barking, whining, growling, and howling
Yawning	F	Slow and deep inhalation with forced and involuntary jaws and mouth opening

Salivary cortisol assessment

Salivary cortisol concentration (ng/mL) was assessed on dogs under basal conditions 15 minutes after arrival at the facility (T0), 22 minutes after the end of each reading session (T1), to capture post-session levels, which correspond to the time during AAA sessions, and 22 minutes after T1 (T2).

In addition, saliva was collected at similar times as in reading days (4:00 pm, 5:05 pm, 5:27 pm) during 2 nonconsecutive control days from the dogs. To avoid potential effects of food or exercise on home baseline cortisol, the dogs were not fed at least 1 hour before sampling and did not undergo any hard or unusual exercise on that day (Glenk et al., 2014).

All samples were taken by the handler using Salivette Cortisol tubes (Sarstedt, Nümbrecht, Germany). The swab was gently placed into the cheek pouch or under the tongue of the dog for approximately 30-50 seconds, without restraint of the animal. The dog's salivation was stimulated by smelling food treats. The dog received a food treat only after the saliva sample was taken (Bennett & Hayssen, 2010; Ligout, 2010). Each sample was replaced in the device tube and closed with a plastic stopper to avoid evaporation. The collected material was refrigerated at -4°C and then stored at -20°C. At the time of analysis, the samples were thawed at room temperature and centrifuged (3500-rpm for 15 minutes).

Analysis was performed using a multispecies cortisol enzyme-linked immunosorbent assay kit (DiaMetra S.r.l., Milano, Italia), according to the protocol for salivary samples. The intra- and inter-assay coefficients of variation were ≤10% and ≤8.3%, respectively. The mean minimum detectable dose was 0.12 ng/ml.

Statistical analysis

Data were analysed through nonparametric statistical tests. Differences in cortisol levels and behaviors between time points and dogs and within time points were analysed using Kruskal-Wallis test for multiple comparisons and One sample Pearson's chi-square test, respectively. Post hoc Mann-Whitney U tests with the Bonferroni correction followed Kruskal-Wallis test in case a significant effect was detected.

Cortisol concentrations, duration, frequency and relative frequency of behaviors are presented as mean ± SE and/or percentage. P values ≤0.05 were deemed statistically significant. Statistical analyses were performed with SPSS, version 25,0 (SPSS Inc, Chicago, IL).

Results

For both dogs, the proportion of time spent exploring and resting was similar during each observation period (Table 2), and no significant differences were found among the phases. Dogs spent more time in recumbent rest than standing either before (Kruskal-Wallis $\chi^2 = 10.487$, df = 2, P = 0.009) or during the reading sessions (Kruskal-Wallis $\chi^2 = 40.021$, df = 2, P = 0.001) (Fig. 2, A-B).

Table 2. Mean duration of dogs' non-stress behaviors expressed as percentage.

Subject	Phase	Behaviors	Duration (%)
Lilly	pre	Resting	41.39
Lilly	pre	Exploring	56.43
Lilly	pre	Looking at children	0.48
Lilly	pre	Looking at handler	1.38
Lilly	during	Resting	45.69
Lilly	during	Exploring	46.29
Lilly	during	Looking at children	6.75
Lilly	during	Looking at handler	1.27
Lilly	post	Resting	57.79
Lilly	post	Exploring	41.15
Lilly	post	Looking at children	17.55
Lilly	post	Looking at handler	0.66
Bella	pre	Resting	20.15
Bella	pre	Exploring	64.20
Bella	pre	Looking at children	7.62
Bella	pre	Looking at handler	8.04
Bella	during	Resting	33.18
Bella	during	Exploring	52.45
Bella	during	Looking at children	8.02
Bella	during	Looking at handler	6.35
Bella	post	Resting	54.66
Bella	post	Exploring	44.72
Bella	post	Looking at children	0.62

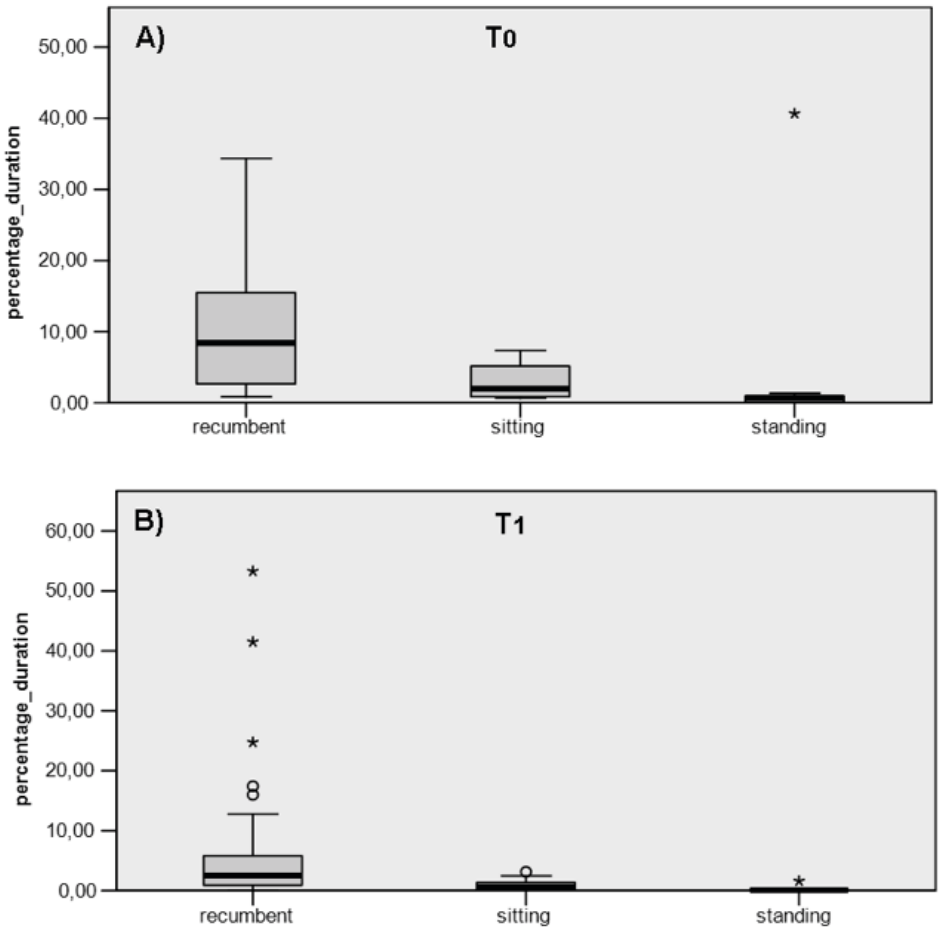

Fig. 2. Percentage of time spent in resting activities in T0 (A) and T1 (B) by the two dogs.

Stress-related behaviors were rarely observed in both dogs. Tail down was the most frequent stress-related behavior before (One sample Pearson's $\chi^2 = 38.000$, df = 3, P = 0.001) (Fig. 3A) and during (One sample Pearson's $\chi^2 = 270.423$, df = 10, P = 0.001) (Fig. 3B) the reading sessions in both dogs.

Summing the behavioral signs of stress, we found no significant between-subject differences in terms of relative frequency in each observation period.

No significant differences were also found in the relative frequency of lip licking (Table 3).

No between and within-subject difference was observed in cortisol values during control days. Bella had higher salivary cortisol levels in T0 than in T2 (Kruskal-Wallis test, $\chi^2 = 8.703$, df = 2, P = 0.013) during reading days (Table 4). Between the dogs, Bella had significantly higher salivary cortisol levels than Lilly in T0 (Table 4) and in T1 in reading days (Kruskal-Wallis test, $\chi^2 = 5.000$, df = 1, P =0.025 and $\chi^2 = 4.083$, df = 1, P = 0.043).

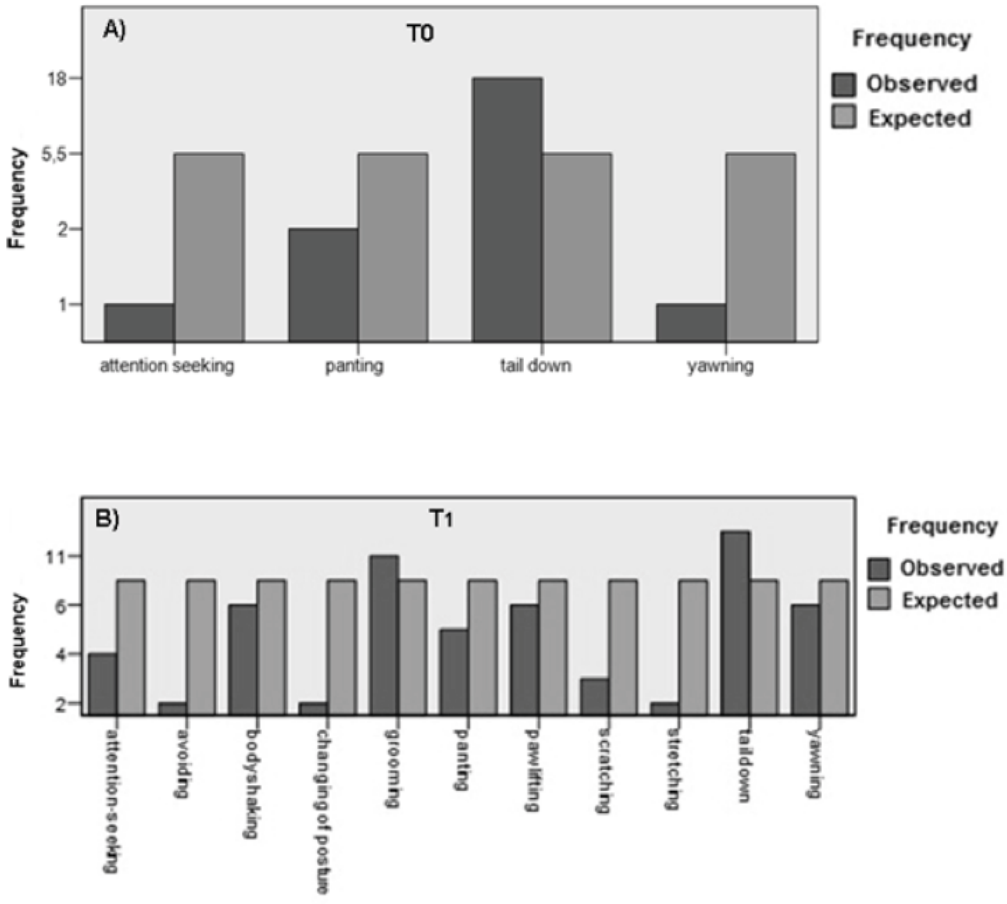

Fig. 3. Differences in the frequency of stress-related behaviors exhibited before (A) and during (B) the reading session by the two dogs.

Table 3. Relative frequency of dogs' stress behaviors and lip licking before, during, and after the reading sessions.

Subject	Phase	Total time (sec)	Stress behaviors (Freq/min)	Lip licking (Freq/min)
Lilly	Pre-reading	870.10	1.31	4.00
	During reading	10534.20	0.57	0.47
	Post-reading	608.20	1	0.98
Bella	Pre-reading	399.60	0.45	3.00
	During reading	8565.74	0.15	1.28
	Post-reading	462.30	0.39	0.65

Freq= frequency; min= minute

Table 4. Mean concentrations of salivary cortisol (ng/mL) in dogs at T0, T1 and T2 during control and reading days.

Subjects	Days	Cortisol (ng/mL)					
		T0		T1		T2	
		Mean	SE	Mean	SE	Mean	SE
Bella	Control	1.114	0.172	1.929	0.175	2.013	0.154
Bella	Reading	29.021*§	4.990	3.535**	0.800	2.694	0.320
Lilly	Control	0.275	0.213	0.092	0.024	0.319	0.015
Lilly	Reading	0.673	0.407	1.136	0.547	1.061	0.550

SE=standard error

* P = 0.025 *vs* the other dog's T0 during reading days, Kruskal-Wallis test

** P = 0.043 *vs* the other dog's T1 during reading days, Kruskal-Wallis test

§ P = 0.013 *vs* T2, Kruskal Wallis test

Discussion

This case study aimed to contribute to the limited scientific research into dogs' welfare during reading-to-a dog's programs. Using a field-based methodology we investigated the effects of participation in reading sessions with PPD children on the behavior and salivary cortisol concentrations in two dogs.

The rationale for implementing reading sessions at the presence of a dog is that it indirectly contributes to improving children reading abilities and learning experiences (Genlott & Grönlund, 2013) by increasing children motivation to read (Shaw, 2013). Although the use of reading to dogs programs is a growing phenomenon in a wide range of educational services, before this practice is recommended for implementation into mainstream education, further scientifically rigorous research is needed to quantify the effects on both the children and the dogs during this particular activity using standardised endpoint-based analysis (Pirrone et al., 2017).

According to the initial hypothesis, in our study, the dogs showed no physiological or behavioral changes indicating stress during the reading sessions. Salivary cortisol levels were determined to be no different between control and reading settings. Overall, findings suggest that this particular activity, or expectation itself, did not negatively affect their welfare. This was likely because activities were predictable and controllable. Environmental factors, loud noises, exposure to novelties (e.g., children, voices) are all potential stressors in this context. However, social interactions are the most potent stressors a dog can endure (von Holst, 1998; Karatsoreos & McEwen, 2011) because they may be uncontrollable, requiring the individual to constantly adapt physiologically and behaviorally to maintain homeostasis (Karatsoreos & McEwen, 2011). Moreover, forced positions in which animals cannot avoid invasive social intrusions and do not have the opportunity to seek refuge may impair their welfare (Hatch, 2007; Piva et al., 2008; Serpell et al., 2010; Glenk et al., 2013). For this reason, the facility room, was set up to allow only safe and predicable interactions between the children and the dogs, without children-initiated physical contacts. It should be noticed that, except for one occasion, the dogs, for their part, never voluntarily chose to establish physical contacts with the children and other people in the room.

It is worth noticing that one dog showed significantly higher cortisol levels than the other dog in T0 on reading days. Moreover, values were even higher than in T1 and T2, when they were always in normal range (0.70 – 3.40 ng/ml) (Sandri, 2015). This might suggest that this dog was less confident with car transport itself, rather than with the social environment provided by PPD children reading to her. Accordingly, a recent study by Radisavljević et al. (2015) reported higher

glucose concentration, leukocyte and neutrophil counts, neutrophil/lymphocyte ratio and cortisol, among other markers, immediately after the car transport in 40 free-roaming dogs compared to the levels of the same parameters detected in the dogs after housing in the new environment.

This also means that, as with other canine-assisted activities, the suitability of untrained dogs should always be discussed in the reading to dogs' plan. Despite the widespread belief of dog owners that their animals are ideal companions, this does not necessarily make them good candidates for such interventions. Some AAI (Animal Assisted Intervention) programs involved shelter dogs with the aim of enhancing socialisation and adoption rates (Hatch, 2007). Based on interviews with human volunteers in an animal shelter's Animal Assisted Activity (AAA) program and participant observation in the same program, Hatch (2007) investigated the animals' experience in AAA, concluding that these programs raise numerous concerns for the animals involved.

In our study, the frequency of stress signs was quite low in the two dogs. However, they exhibited some stress-related behaviors before and during the reading sessions, particularly a lowered position of the tail, which could actually suggest some insecurity. Dogs also showed some lip licking, especially on arrival at the facility, which we decided to analyse separately from the other stress signs because of the potentially contradictory meaning it may convey (Albuquerque et al., 2018).

One of the most recently debated issues is whether lip licking, similarly to other subtle cues (e.g., yawning, panting, body shake, paw lifting), actually represents stressful conditions in dogs. On the one hand, lip licking has been shown to precede situations of social conflict (Voith et al., 1996) or, in guide dogs, to be displayed by dogs that performed poorly on a task (Tomkins et al., 2011). On the other hand, according to Rehn & Keeling (2011), lip-licking may be a communicative cue in dogs, which does not necessarily correspond to a stressful experience but, on the contrary, may help to manage stress. This behavior has been attributed to increased positive arousal and affiliative social interaction (Rehn & Keeling, 2011; Shiverdecker, 2013), being observed among wild dogs approaching peacefully conspecifics (Meyer, 2006) and as a common component of the greeting behavior of dogs toward humans (Overall, 2013; Feddersen Petersen, 2008).

Overall, our findings, together with these considerations, indicate the need for more attention to the experience of animals in reading to dogs' programs.

Although this report refers to two subjects, Lilly and Bella, the results of our work represent an encouraging basis for further studies on a wider scale. Our dogs, besides their ability to adequately adapt to the reading-to-a dog context, showed expected benefits, particularly in terms of successful re-homing. The analysis of the benefits for children from reading to dogs' sessions comprises another part of the multidisciplinary project and are not reported on here. In brief, many positive effects of Bella and Lilly's presence were recorded.

In conclusion, participation in the program did not result in any welfare concerns for the dogs. No significant physiological or behavioral indicators of stress, fatigue, or exhaustion, in fact, could be detected during safely conducted 30 minute-reading sessions with PDD children. However, the small sample size of this study requires non to generalise. Further studies with a larger sample size and covering more sessions, with more children, are needed for more generalised results, as well as to explore the possible effects of either dogs' or environmental-related factors on canine well-being and performance.

In line with the literature, and according to our preliminary results, dogs should be considered when planning these interventions for PDD children and their welfare should be monitored continuously. Considering that dogs may not exhibit stress-associated behavior in the context of human-animal interactions (Kuhne & Hößler, 2012) despite being physiologically stressed (Ng et al., 2014), behavior should always be assessed in conjunction with physiological parameters, such as cortisol in saliva.

Ethics statement

The study was performed according to the Declaration of Helsinki. The ethics committee of the CTR Onlus approved the study, protocol number CR3/403-18, 2018. No invasive intervention or drug experimentation non the dogs was performed; therefore the application of Legislative Decree No 26/2014 and Directive2010/63/EU for the protection of animals used in scientific and experimental studies was not required. The Effetto Palla Onlus provided consent for use of the dogs in the study. Participants were recruited between September 01, 2017 and October 01, 2017.

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Valutazione del comportamento e dello stress in due cani durante sessioni di "lettura al cane" in un programma per bambini affetti da disordini dello sviluppo

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Sintesi

Studi precedenti suggeriscono che vi sia un miglioramento nella capacità di leggere e nell'attitudine alla lettura quando i bambini leggono in presenza di un cane. Questo fenomeno sembra essere correlato al fatto che il cane sia in grado di agire come un ascoltatore attivo e che fornisce supporto. Tuttavia poco si conosce sulle implicazioni per il benessere dell'animale che queste attività hanno. Sebbene i cani ricevano sostegno e conforto durante le sessioni di attività assistita, essi potrebbero sperimentare stress che potrebbe causare una riduzione della motivazione a lavorare e della loro prestazione.

Il cortisolo salivare e i comportamenti sono stati analizzati in 2 cani, in buono stato di salute, durante e dopo 30 minuti di una sessione di attività assistita di lettura con 4 bambini con disturbi dello sviluppo, per identificare possibili segni di stress.

Sebbene uno dei cani avesse livelli di cortisolo salivare significativamente molto alti, al momento del suo arrivo nella struttura, nessun segno fisiologico o comportamentale di stress fu rilevato nei cani durante e dopo le sessioni di attività.

In conclusione, questo tipo particolare di attività non ha influito negativamente sul benessere del cane.

Ulteriori studi con un campione più ampio sono necessari per esplorare più accuratamente sia i benefici che i bambini affetti da disturbi dello sviluppo possono ottenere da queste attività, sia lo stato fisiologico del cane, nella prospettiva di "One Health - One welfare".