Dog Behavior, 2-2021, pp. 39-54- • DOI 10.4454/db.v7i2.140

Stress assessment of co-therapist dogs in animal assisted interventions: a review

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Abstract: Animal assisted intervention (AAI), in their three forms (Animal-Assisted Activities, Animal-Assisted Education and Animal-Assisted Therapy), are activities aimed not only at supporting and integrating traditional therapies, but also at developing a strong link between animal and human beings in non-therapeutic contexts.

Despite the benefits of these activities on humans, demonstrated by several studies, it is important not to underestimate the risks for the animals involved, especially dogs. These risks are represented by different types of stress, mostly acute, which, if repeated over time, could lead, in the long- time period, to a burnout syndrome in the animal.

The analysis of scientific literature's review of recent years, regarding the various publications on the assessment of stress in the dogs involved in the AAI, has confirmed that it does not seem to be great concerns about the welfare of co-therapists if certain practices are avoided and if considered the various factors (environmental, human and program management) that can influence it.

However, for the future, it would be desirable to aim at a further exploration of the canine experience within the AAI, using validated and standardized scientific instruments.

Key Words: AAI, dogs, stress, behavior, literature review.

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Introduction

Animal-assisted interventions (AAI) represent a widespread reality for more than thirty years and are reaching a certain level of recognition all over the world and this is also thanks to the multiple researches on the effects that these activities can have on human health and welfare. Animals are believed to be a strong motivation for people to take part in health interventions, exercise and social interaction (Glenk *et al*., 2014).

The widespread involvement of dogs in AAIs is based on the exceptional inter-specific social capacity of this species and the easiness with which they adapt to the multiple situations that characterize the human environment (Miklosi *et al*., 2013).

The results of these interventions are now scientifically validated by multiple experiments, carried out in different fields of application: from the psychological welfare of university students to the reduction of pain in hospitalized children, to helping children to improve their reading skills, to modifying the behavior of children with autism, to reducing anxiety in hospitalized patients and improving the quality of life of patients suffering from dementia (de Carvalho *et al.,* 2019).

Although in Italy the number of scientific publications, attesting the therapeutic efficacy of animal-assisted interventions is still small, the results of some research projects are extremely encouraging.

Among the species employed in animal-assisted interventions (dog, cat, horse, donkey and rabbit), the dog is certainly the most often chosen as a co-therapist as it stimulates attraction and emotional involvement in humans in a very natural way (Cole & Gawlinski, 2000).

However, it is essential to understand that welfare must be mutual; clinical and psychological welfare in humans cannot be produced if the animal is also not in optimal psycho-physical conditions. For this reason, particular attention must be paid not only in the selection phase of the animal to be employed, but for the entire duration of the intervention and for the entire "career" of the dog.

Submitted, 12/22/2021
Accepted, 01/20/2022

In AAIs, are normally involved adult, male and female dogs that are not in estrus, lactation or advanced pregnancy; about the breed, they can be employed both pure and mixed breed dogs, which have characteristics of sociability, docility, good intra- and inter-specific relational skills and above all that do not present health as well as behavioral problems (aggressiveness, hyper-activity, excessive shyness, phobias, anxiety, etc.). The behavioural requirements of dogs must be monitored during the AAIs in the manner indicated by the veterinarian and periodically verified. In addition, at the end of the AAI project, it is necessary to carry out a new assessment of the welfare status and of any other behavioral change of the animal.

Despite the potential benefit of AAIs, the risks for both humans and dogs involved should not be underestimated, such as the possible transmission of zoonoses or dogs' burnout syndrome, which is a major concern about their welfare.

As stated by Marinelli *et al.* (2009), the growing demand for AAIs means that there is a greater demand for sessions, with the participation of increasingly large groups; this undermines the quality of the interventions themselves, compromising the welfare of dogs involved, due to over-exposure to stressful situations for the animal, such as activities in unknown environments, too close interactions with unknown people and unforeseen situations.

Although many data support the thesis of the benefits of these activities for humans, there are still few studies that have focused on the possible welfare implications for dogs as a result of their performance in AAIs. In fact, the social interaction of dogs during AAAs and TAAs represents one of the most powerful stressors that a dog can endure and probably this is why the social interaction is certainly unpredictable and requires constant physiological and behavioral adaptation by the animal in order to maintain homeostasis (Palestrini *et al.*, 2017).

Further studies about the psychological and behavioural effects of AAIs on dogs involved are needed, in order to increase knowledge about animal welfare during these interventions, to introduce new evidence-based guidelines for animal handlers and to establish rigorous methods for future research.

Welfare and stress in dogs

Animal welfare can be defined as the animal's ability to adapt to and cope with the demands imposed by its environment (Broom & Fraser, 1990) and the freedom to express a natural behavior is an important aspect of dog's welfare (Glenk *et al.,* 2014).

About the concept of stress, the Austrian physician Hans Selye was the first in 1936 to take an interest in the concept of stress in his research, describing it as a non-specific response of the organism to a harmful stimulus. Afterwards, many other authors have given different definitions, but all of them describe it as a response of the organism to an internal or external threat in which it concentrates its effort to deal with it and to restore the original state. Selye distinguished stress in *eustress* (positive or physiological stress), at the base of which there is a normal and highly adaptive mechanism that allows the animal to react quickly to an event that changes its homeostatic status and *overstress* and *distress* (negative stress) where the response to it becomes problematic and therefore the animal is not able to control the situation or to escape the stressor through an appropriate behavioral response and the adaptive effort requires the expenditure of considerable energy at the expense of other important biological functions (for example, growth and reproduction) of which the animal can (distress) or can not (ovestress) be aware.

Stressors can be divided into: physical or physiological, psychological and social (conspecific behaviors, overcrowding, isolation); according to duration they can be divided into acute, intermittent and chronic.

Physio-pathology of stress

Whatever the stressful event, the animal implements four types of responses:

1. the active behavioral response that consists in avoiding or facing the cause of stress, the so-called fight and flight reaction. This represents the state of alert;
2. the faster response mediated by the sympathetic nervous system (ANS) with the release, by the adrenal medulla, of catecholamines (adrenaline and norepinephrine). Adrenaline is responsible for the increase in heart rate, blood pressure, hydrolysis of glycogen into glucose and therefore the energy available to cope with stress (state of resistance);
3. the slower response, mediated by the hypothalamic-pituitary-adrenal axis, an essential component for the control of the neuronal, endocrine and immune response. Hypothalamic neurons produce the corticotropin-releasing hormone/factor (CRH/CRF) and the arginine vasopressin hormone (AVP); CRH modulates the action of corticotropin-producing cells located in the pituitary gland with the consequent increase in synthesis and release of adrenocorticotropic hormone (ACTH), which induces the release of glycocorticoids by the adrenal cortical. This also leads to an increase in the blood concentration of catecholamines, which will act as neurotransmitters at the level of the amygdala and hypothalamus, important brain areas involved in processing the potential danger of the stressful stimulus (Panzera *et al.*, 2008).
   The glycocorticoids produce effects that can be summarized in:stimulation of protein catabolism in muscle; stimulation of hepatic gluconeogenesis; hyperglycemia; stimulation of fat catabolism; inhibition of pituitary gonadotropins; inhibition of pituitary thyrotropin; inhibition of pituitary growth hormone; depression of the immune system. They also act centrally, at the level of the hypothalamus and higher nerve centers, in particular hippocampus, with a negative feedback mechanism in order to prevent the continuous activation of the hypothalamic-pituitary-adrenal axis.
4. The immunological response, with effects on the immune system. The connection between the hypothalamic-pituitary-adrenal axis and the immune system is demonstrated by the action of glycocorticoids which act by altering the inflammatory response by inhibiting the release of IL-12 and causing a dysregulation of lymphocytes; they also cause marked neutrophilia with reduction of lymphocytes, eosinophils, basophils and monocytes. Finally, at high concentrations, they can interfere with the production of immunoglobulins. Catecholamines may be responsible for a dysregulation of lymphocytes and the alteration of the tumoricidal and antiviral activities of macrophages. CRH factor is responsible for increasing IL-1 and reducing the cytotoxic activity of natural killer cells; adrenocorticotropic hormone (ACTH), besides stimulating the production and release of glycocorticoids, stimulates the proliferation of B lymphocytes. Among the hormones produced, the Growth Hormone (GH) stimulates the production of antibodies and the activity of natural killer cells, while prolactin stimulates the production of lymphocytes and β-endorphins which inhibit the immune function (Berteselli *et al.*, 2006).
   At the state of resistance, if the animal does not find a balance, it remains in a state of malaise and it is exposed to any other harmful cause that may intervene (state of exhaustion).

Stress indicators in the assessment of dog welfare

In accordance with the scientific literature, the most used parameters for the stress response evaluation in dogs are represented by behavioral and physiological markers (Kooriyama & Ogata, 2021).

Stress-related behavioral markers are nothing more than normal adaptive responses that allow the animal to return to the homeostatic state. These responses can become problematic when the dog is no longer able to adapt and above all to escape the stressful stimulus (chronic stress conditions). Behavioral markers vary greatly from individual to individual and their measurement has the advantage of not being invasive; it also allows to evaluate individual responses. They are mostly represented by: agitation, panting, environmental exploration, mydriasis, pacing, vocalizations, eliminations, tremors, avoidance, nose and lips licking, facial expressions, level of activity (Berteselli *et al.,* 2006; Bremhorst *et al.,* 2019; Cannas *et al.*, 2014; Cozzi *et al.*, 2016; Csoltova *et al.*, 2017; Mariti *et al.*, 2017; Kooriyama & Ogata, 2021). The evaluation of behavioral markers is certainly faster than that of physiological ones, as dogs' body language and facial expressions can be used instantly to determine their emotional state (Kartashova *et al.*, 2021; Mota-Rojas *et al.*, 2021). There is still no scale accepted by all researchers for the visualization of stress. From the analysis of several articles it is clear that there are terms and descriptions of some behaviors that are common, others instead are mentioned only in some studies. Kartashova *et al.*, after analyzing the various tables with signs to determine stress in dogs, created a summary (Tab. 1).

Tab. 1. General behavioural markers of stress in dogs (from Kartashova *et al.,* 2021).

General behavioral markers of stress in dogs.

	Stress and negative emotional state	Low stress level, no stress
General condition	Anxious/hypervigilant (C, F, G), shaking/trembling (B, C, G, F), obviously very tense (but can be cooperative) (C, G, H), impossible to sample (E), impossible to touch (E), needs to be lifted up or brutally forced when pulled by the leash (C, G), difficult to maneuver on leash and encouragement doesn't help (C, G), looking/acting sleepy (when not tired) or distracted (G), refusing to eat (H).	Low stress: alert, but calm and cooperative (C, G). No stress: extremely friendly (G), outgoing (G), solicitous of attention (G), calm (C, G), relaxed (C, G), seemingly unmoved (C, G).
Behavior	Moving away (E, C, G), hiding (C, D, G, F), refusing to sit/lie down (C, G), walking back and forth (B, D), not remaining in one place (D), cowering (B), attacking (E), biting (E, F), snapping (F), destroys/scratches/chews at surfaces (windows, doors, carpeting, furniture) (D), circling (B), panting (B, C, F, G), yawning (B, D, F, G), eliminating (B, D, F), spitting (E), refusing treats (G), sniffing (G), attempting to jump off the table (F).	
Body and Head	Low head position (F),side-turning of the head (B, E), turning the head away (E), whale eye (F,G), ear drive (A), flattened ears (A), licking nose or lips (A, D, F, G), lifted lip (E), tongue out (B), dropped jaw (A), swallowing a lot (D), lifting or withdrawing paws (E), raised hair (E), salivating (G), skin and muscles visibly moving (D), tucking the tail (B, F).	
Vocalization	Barking/howling (B, C, D, G, F), growling (E, F), whining (B, C, D, G), whimpering (D).	

(A) (Bremhorst et al., 2019).
(B) (Gutiérrez et al., 2019a).
(C) (Hernander, 2008).
(D) (Dreschel and Granger, 2005).
(E) (Srithunyarat et al., 2018).
(F) (Scalia et al., 2017).
(G) (Lloyd, 2017).
(H) (Mariti et al., 2017).

Physiological markers, on the other hand, provide quantitative measures and therefore allow to obtain values that can be compared with others, in different individuals and under different conditions. The most used over time have been: cortisol in the peripheral blood and saliva, heart rate, body temperature or a combination of them (Bergamasco *et al.*, 2010; Csoltova *et al.*, 2017; Hennessy, 2013; Polgàr *et al.*, 2019; Rigterink *et al.*, 2018; Wormald *et al.,* 2016; Ogi *et al.*, 2020). In the most recent studies, other markers, such as Chromogranin A and salivary alpha amylase, some inflammatory cytokines, C-reactive protein, haptoglobin, secretory immunoglobulin A, cathestatin and prolactin, have also been taken into account (Kartashova *et al.*, 2021; Kooriyama & Ogata, 2021).

1. Blood cortisol is certainly one of the most used markers to study the response of the hypothalamic-pituitary-adrenal axis.It is generally assumed that an increase in its concentration is a clear indicator of stress in the animal; however this is not always the case, because it has been seen that, chronic stress can lead to a depletion of energy reserves and therefore to a reduction in blood levels of cortisol, as the adrenal gland is not able to maintain its concentration at certain levels (Kartashova *et al.*, 2021; Righi *et al.*, 2019). In addition, the method of collecting samples also af-

fects the concentration of blood cortisol, since, being collection method invasive, it can cause pain and acute stress and a temporary increase of the same in the blood (Corder-Ramos *et al.*, 2019; Kartashova *et al.*, 2021; Righi *et al.*, 2019).

Recently, in some studies, salivary cortisol has been used as a marker of acute stress, as well as the concentration of cortisol in the feces and hair (Mariti *et al.*, 2020), although, regarding the latter two, the studies are still few and there are not many data to compare (Kartashova *et al.*, 2021). However, these few studies have found that the concentration of cortisol in the dog's hair can be a good indicator of chronic stress (Heather *et al.*, 2013; Packer *et al.*, 2019). In addition, the immunoreactivity of cortisol in hair has been shown to be less variable than in saliva and feces (Heather *et al.*, 2013). What is more, it has been found that age, pregnancy, hair color, body region, gender, and season of year may have a reflection on the concentration of cortisol in the hair (Heimbürge *et al.*, 2019). At the moment, there is not enough evidence to conclude that the concentration of cortisol in the hair accurately reflects the long-term concentration of this hormone in the blood, although it is a good parameter to be studied in the future for the assessment of chronic stress in the dog.

Recent studies on the evaluation of cortisol levels in dog cut nail samples have also shown that there are correlations with levels of the same hormone in the hair (Kooriyama & Ogata, 2021; Mack & Fokidis, 2017).

About salivary cortisol, since the hypothalamic-pituitary-surrenal axis responds more slowly than the autonomic nervous system, if this parameter is used alone, the study must be carefully designed, as the intensity of the stressor and sampling times affect the results (Kooriyama & Ogata, 2021).

Salivary cortisol has certainly been the most frequently used parameter in recent studies, but the intra- and inter-individual variability is very high and the results of the various studies are not entirely coherent (Escribano *et al.*, 2013; Kanai *et al.*, 2008; Kooriyama & Ogata, 2021; Ryan *et al.*, 2019); in fact, several factors can influence the concentration of cortisol, both salivary and blood, such as: individual differences in physiological responses to stress, age, sex, race (Höglundet *al.*, 2016), the type of relationship with the owner (Kartashova *et al.*, 2021; Schöberl *et al.*, 2016; Svobodova *et al.*, 2014; Wojtas *et al.*, 2016), excessive exercise, the methods of transport of the animal and its reaction to new environments. For this reason, despite its popularity as a parameter in assessing stress in dogs, according to Kooriyama *et al.*, salivary cortisol cannot be considered as the only salivary parameter to be studied but it would be advisable to use multiple parameters that can study both the autonomic nervous system (short-term response) and the hypothalamic-pituitary-surrenal axis (long-term response); this would allow more information on the individual response to stressors in the appropriate settings (Lensen *et al.*, 2019).

2. The increase in heart rate and the reduction of heart rate variability are physiological responses to stress and are used as parameters when making a stress assessment in the dog; these are standard indicators that can easily be evaluated, with the sole indication that the animal has no other concomitant cardiovascular problems (Kartashova *et al.*, 2021). Moreover, these parameters should be used in combination with others, since, in addition to stress, other conditions can also lead to an increase in heart rate, for example interaction of the animal with the owner and a stranger (Palestrini *et al.*, 2005) or intense physical activity.

3. Other potential salivary parameters to consider in the assessment of acute stress include Chromogranin A (CgA) and alpha amylase (sAA).

CgA is a newly discovered acidic protein that is stored in the secretory granules of endocrine, neuroendocrine and neuronal cells. Salivary CgA in animals (dogs and pigs) is produced (activity mediated by the action of catecholamines) and stored in the salivary glands; it, therefore, is considered a useful parameter for the evaluation of the activity of the sympatho-adreno-medullary system (acute stress). In human medicine, CgA levels in saliva depend on circadian rhythm, peaking upon awakening, decreasing within an hour and then remaining low throughout the day; in dogs, instead, the salivary CgA levels measured every four hours do not seem to have undergone circadian variations, which could represent an advantage for the interpretation of this analyte in this species (Kanai *et al.,* 2008; Kooriyama & Ogata, 2021). Ryan *et al.* (2019) collected saliva samples in dogs to measure CgA and cortisol levels before and 20 minutes after the Strange Situation Test (SST), and deduced that CgA levels of all dogs decreased in samples collected after SST, but cortisol increased in 40% of dogs and decreased in 60% of dogs compared to samples collected before SST;the authors explained the decreased levels of CgA in relation to the timing of collection of salivary samples, as CgA could have peaked during the introduction of the animals to the test site and then decreased rapidly and significantly before the second sampling (after the SST). In dogs, unfortunately, there are not yet many studies on the evaluation of salivary CgA levels, therefore, interpreting CgA levels in dog saliva still requires caution and other studies, when used in combination with other parameters (e.g. cortisol), but for sure CgA levels reflect the activity of the sympatho-adreno-medullary system and could certainly help in assessing stress in dog.

About salivary alpha amylase (sAA), recent studies have confirmed the presence of this enzyme in dogs, especially at the level of the submaxillary glands, but its concentration is very low compared to humans. Contreras-Aguilar *et al.* (2017) measured sAA levels in dogs before and after sympathetic activation, which typically corresponds with the stress reaction, and these were increased after such activation, thus supporting the use of sAA as a non-invasive parameter to measure stress in dogs; moreover, another advantage of the use of sAA is that it remains stable in samples stored for at least 6 months at -20°C without the use of preservatives (Ricci *et al.,* 2018; Kooriyama & Ogata, 2021).

4. Although there are not many studies on dogs, but many on humans, it would seem that inflammatory cytokines, in particular some interleukins and a type of TNF (tumor necrosis factor), could prove to be good parameters for assessing stress in this species.

In pigs, interleukin 18 (IL-18) has been measured during stress due to immobilization and it seems that it is more sensitive than salivary cortisol to the activation of the sympathetic nervous system, therefore it would seem that it represents a good salivary marker for the study of acute stress (Muneta *et al.,* 2014).

5. About the reactive C protein and haptoglobin, major proteins of the acute inflammatory phase studied in humans, but little in animals, even if they have never been used as stress parameters in dog saliva (as instead happens for the serum of these animals), they could be valid markers, also because there are studies that confirm a correlation between serum and salivary levels of these two proteins in dogs (Cho *et al.*, 2020; Kooriyama & Ogata, 2021; Parra *et al.*, 2005).

6. Secretory immunoglobulin A (sIgA) is another proposed parameter, used together with glycocorticoids, to assess animal welfare status (Srithunyarat *et al.*, 2018). It is a dimerproduced by B cells distributed in the oral (salivary glands), tracheal and intestinal mucosa and represents the main immune defense at this level (Brandtzaeg, 2013). Studies, in which cortisol and sIgA levels were evaluated at the same time, revealed that the interpretation of the results is somewhat complicated due to several factors, such as age. In fact, it has been seen that in puppies, after acute stress, there is an increase in the concentration of sIgA, while in the saliva of adult dogs there seems to be a reduction (Svobodova *et al.*, 2014). To better understand the correlation between the levels of this immunoglobulin and the age of the dog, more research is needed.

In addition, according to studies by van de Velde *et al.* (2012), it seems that physiological factors such as a sharp increase in body weight can also cause an increase in the concentration of sIgA.

Measuring salivary sIgA levels during a period of tranquility in the animal has shown that there is considerable variation in daily rhythm, with an increase in the morning and subsequent reduction throughout the day (Kikkawa *et al.*, 2003).

In another study, conducted by Lensen *et al.* (2019), the relationship between cortisol, chromogranin A and sIgA levels in puppies was analyzed and the authors came to the conclusion that sIgA itself is not an objective parameter for stress assessment, but in combination with cortisol and other physiological and behavioral parameters, it is possible to develop a good scheme to interpret the stress level. In conclusion there is a need for further studies to understand the real correlation between stress and sIgA levels.

7. Catestatin is an endogenous, cationic and hydrophobic peptide, which derives from the proteolytic clevage of its precursor, chromogranin A (Mahapatra, 2008). It is considered among the new parameters for assessing stress. In fact, it seems that its concentration in saliva increases together with the level of cortisol in the serum of dogs evaluated for stress (Srithunyarat *et al*., 2018). In the study of Srithunyarat *et al.* (2018) serum catestatin levels are not much different between the group of dogs under study (unaware of sampling procedures) and the control group (accustomed to sampling procedures). However, another study by the same author (2017), suggests that, in dogs with bone fracture, changes in serum catestatin concentration, before and after morphine administration, may represent a good clinical parameter for assessing pain-related stress.

8. Prolactin seems to play a very important role in the central nervous system, as it preserves the hippocampal neurogenesis of adults during chronic stress, counteracting the antiproliferative action of glycocorticoids during the early stages of stress, promoting neuronal growth and differentiation or supporting the survival of newly generated cells after continuous exposure to stress (Lensen *et al.*, 2019; Torner *et al.*, 2009). The concentration of serum and salivary prolactin in dogs has been proposed (Gutierrrez *et al*., 2019 a,b) as a parameter in the evaluation of both acute and chronic stress, but the results of the same do not seem to support the hypothesis, also because the ELISA kit, used to measure serum prolactin levels, is not reliable to measure salivary concentration; moreover, in many cases the salivary concentration of prolactin is below the limit value (Gutierrez *et al.*, 2019; Lensen *et al.*, 2019). Therefore, other studies are certainly needed to validate the use of salivary prolactin as a parameter for assessing stress in dogs.

9. Recently there have been some researches that have also taken into account salivary vasopressin as a possible parameter to be considered in the evaluation of the activity of the hypothalamic-pituitary-surrenal axis (Aguilera & Rabadan-Diehl, 2000; Jeong *et al.,* 2020; Kooriyama & Ogata, 2021; Pirrone *et al.*, 2019). The results of the evaluation of vasopressin levels in dog saliva demonstrate the opposite response of this hormone and oxytocin to stressor stimuli, but no significant changes in oxytocin levels have been seen, probably due to the different types of stressors or the different measurement methodologies used. Therefore, it is not yet clear whether the peripheral concentration of these hormones reflects their activity at the central level.

All the parameters mentioned so far, although probably not an exhaustive list, are still to be studied in order to obtain measurement and interpretation methodologies to be validated, taking into account those factors that can influence the results of the studies, such asthe individual characteristics of the animals studied (e.g. number and location of salivary glands in different species, sex (Sandri *et al.*, 2015), age (Chmelìkovà *et al.*, 2020), sampling methodology and products used to stimulate salivation (citric acid, food or food smell)(Damiàn *et al.*, 2018). In conclusion it is possible to say that, although many of the salivary parameters mentioned, are still being tested, surely those that reflect the activity of the sympatho-adreno-medullary axis and the immune system, combined with cortisol (which reflects the activity of the hypothalamic-pituitary-adrenal axis) have a good potential to provide physiological information on the stress response in dogs.

Clinical studies related to the evaluation of stress in co-therapist dogs

All the authors of the research followed over the years, even if they are still few, agree with the fact that dogs, being widely employed in human society in various activities such as AAIs, are certainly subjected to various types of acute stress (excessive noise, social interactions, exercise, excessive expectations of handlers or recipients) and that, as far as possible, they should be safeguarded from this. To achieve this goal it is necessary to measure and quantify the level of stress, even if stress is a highly subjective phenomenon and influenced by many factors. To quantify the level of stress it is also necessary to use non-invasive sampling methods for the dog; that is why many recent studies have focused their attention on using saliva as a starting sample, as it is a relatively well tolerated method by dogs previously accustomed to it and it is also very simple to instruct handlers on the sampling modality.

In almost all recent studies, the most used physiological marker for the evaluation of acute stress in dogs employed in AAIs is salivary cortisol, alone or combined with other parameters such as the behavioural ones, catecholamines, chromogranin A, secretory immunoglobulin A, salivary IgA, heart and respiratory rate. The most used behavioral markers in the various studies were:

– Postures: sitting, standing, recumbency, walking, exploring;
– spontaneous events: lifting a limb, vocalizations, scratching, tremors, jumps, repeated head movements, stretching;
– oral behaviors: panting, keeping the mouth open, licking lips, licking people or objects, self-grooming.

Some authors have deduced from their studies that the technique of collecting saliva samples from dogs trained to sit on signal to receive a reward is minimally stressful, that it is simple to carry out and that samples can be collected at certain times, which is preferable for behavioral studies that focus on assessing their degree of acute stress (Haverbeke *et al.*, 2008; Sherman *et al.*, 2015). In healthy dogs, the concentration of salivary cortisol is the result of the passive diffusion of free cortisol through the cells of the salivary glands and it is about 7-12% of its concentration in plasma that represents the free fraction (not bound to proteins) in biologically active plasma(Beerda *et al.*, 1996; Vincent *et al.*, 1992).

The methods of sampling, extraction and storage of saliva samples significantly influence the results of research, so it is essential to standardize these procedures to allow a comparison of the results obtained in the different studies.In addition, should be considered possible circadian alterations and individual variables of dogs on salivary cortisol levels during the preparation of the experimental phases, data processing and interpretation of final results.And, precisely because of the complexity of the stress response, the evaluation of salivary cortisol should always be combined with the evaluation of behavioral parameters and / or other physiological salivary markers previously described.

The research of recent years, beyond the type of AAI under study, the behavioral and physiological parameters used and the variables that can influence the results, are all united by a high perception of the quality of the interventions that, therefore, have not been considered particularly stressful for the dogs involved.

What the authors are most concerned about is the desire to outline precise criteria in order to ensure the welfare of dogs, as well as other animals, involved in these activities and therefore to create standardized guidelines.

Glenk (2017) conducted a literature review (Tab. 2) regarding the current perspectives on the welfare of dogs involved in the AAI, examining, for all the studies carried out from 2008 to 2017, criteria such as:environment, recipientsand characteristics of the sessions (if AAA or AAT and type of program carried out with residential or external dogs).

Tab. 2. Overview of program definitions, therapeutic environment, recipients, dog sample and welfare indicators. (from Glenk, 2017).

References	AAI Type	Program Type	Environment	Recipients	Dogs (*N*)	Welfare Indicators
Haubenhofer and Kirchengast [52,53]	AAA, AAT	Visitation	Hospitals, schools, rehabilitation centers, nursing homes	Adults, children	18	Salivary cortisol, emotions according to handler
Piva et al. [28]	AAA	Resident	Nursing home	Adults	1	Clinical protocol, behavior, fecal and hair cortisol
Marinelli et al. [54]	AAA, AAT	Resident, Visitation	Hospitals, clinics or rehabilitation centers, schools, nursing homes	Adults, children	18	Behavior, handler questionnaire
King et al. [55]	AAT	Visitation	Hospital	Adults, children	21	Salivary cortisol, behavior, handler questionnaire
Glenk et al. [56]	AAT	Visitation	In-patient mental healthcare	Adults	21	Salivary cortisol
Barstad [57] [1]	AAA, AAT	Visitation	Nursing homes	Adults	13	Behavior, handler questionnaire, cognitive test
Glenk et al. [58]	AAT	Visitation	In-patient substance abuse treatment	Adults	5	Salivary cortisol, behavior
Ng et al. [59]	AAA	Visitation	University	Adults	15	Salivary cortisol, behavior
Palestrini et al. [60]	AAT	Visitation	Pediatric hospital	Children	1	Heart rate, behavior

[1] Master thesis.

As demonstrated in the table, the assessment of welfare in dogs involved in AAIs was carried out at several therapy sites including inpatient and outpatient facilities, schools and universities. All studies have provided guidance on the type of AAI performed, whether AAA or AAT or both. In most studies, the recipients were adults organized, mostly, in groups.

Table 3. instead provides an overview of session characteristics and significant results, indicating that the way in which AAIs were conducted varies considerably between studies.

Tab. 3. Characteristics of IAAI sessions including duration, organization of recipients (individual or group), session intervals, and significant results. (from Glenk, 2017).

References	Duration	Single/Group	Intervals	Significant Findings
Haubenhofer and Kirchengast [52,53]	1–8 h	- [2]	Differed from 9–50 sessions/3 months	↑ Salivary cortisol: on working days, during short sessions with high intensity, high frequency of sessions
Piva et al. [28]	20 min	Group	3–4 sessions/week	↓ Stereotypic autogrooming; ↑ play behavior, socialization; ↓ hair cortisol
Marinelli et al. [54]	10–105 min	Single, group	Daily	↑ Stress-related behavior if recipients were children < 12 years; increase in the frequency of sessions and number of recipients across 3 years
King et al. [55]	2 h	Single	Biweekly	No effect of a short time-out session; ↑ salivary cortisol after 60 min; ↑ behavioral signs of stress in dogs < 6 years and/or < 2 years of AAI experience
Glenk et al. [56]	50–60 min	Group	Weekly	No difference between working and resting days; ↓ salivary cortisol in therapy dogs off-lead
Barstad [57] [1]	30 min	Group	Biweekly	No differences in cognitive task performance before 12 weeks of AAIs and pre-post session; no changes in behavioral variables; ↑ responsiveness to commands; ↓ focus on handler
Glenk et al. [58]	55–60 min	Group	Weekly	↓ Salivary cortisol in session 4 and 5; no changes in behavior
Ng et al. [59]	60 min	Group	- [2]	No difference between working and resting days; ↑ salivary cortisol in novel environment
Palestrini et al. [60]	20 min	Single	- [2]	No changes in heart rate or behavior across 20 sessions

[1] Master's thesis; [2] Information not available.

The duration of sessions differs greatly from 10 minutes to 8 hours and there is also great variability in intervals between sessions, with some dogs participating in AAIs on a daily basis, others several times a week, weekly or less.

Indicators of dog welfare are represented by: cortisol (evaluated in saliva, feces and hair), stress-related behaviors, clinical parameters and perceptions of the animal's handler. Stress-related behaviors include licking lips, panting, lifting paw, shaking body, vocalizing, withdrawing, and self-grooming. Behavior was assessed through direct observation rather than using video recordings of the dogs' responses or handler reports. Behavioral sampling was carried out for the duration of the entire session, during one minute after a two-hour work shift or during sequences where the dog was petted for five minutes.Common interaction programs included verbal praise, caresses, gentle scratches, brushing the dog's hair, walking on or without a leash, obeying commands, throwing or hiding toys, and mild exercises.

In the clinical study of Palestrini *et al.* (2017), a therapy dog was employed in 20 AAT sessions in a children's hospital and the sessions were carried out during post-operative awakening, two hours after surgery. The assessment of the stress level was carried out through heart rate recordings and analysis of stress-related behavior, exploration, passive behavior, environmental orientation and interaction with children, with handlers and other people (e.g. staff, parents). Heart rates remained within the physiological range and did not differ whether the children interacted or not with the dog during the sessions. In addition, the dog never tried to withdraw from the intervention and behavioral variables did not vary between sessions. The fact that the dog panted in a statistically significant way was attributed to the relatively high ambient temperature. The authors concluded that participation in the program did not cause welfare problems for the dog.

An assessment of fatigue, in carefully selected therapy dogs by measuring the motivation of dogsin one particular activity, was conducted by Barstad (2014). The dogs were subjected to a cognitive task in which they had to deal with a surprise contained in a closed bucket; the task was assigned both on a rest day and on days before and after intrevention. The performance obtained showed no signs of fatigue, tiredness or lack of motivation as a result of AAI-related activities. The author concluded that dogs' behavior during AAIs and their performance in a cognitive task remained constant over time. Mild symptoms of stress have been observed in animals, and have suggested that it is necessary to supervise and educate recipients but that the benefits that AAIs offer to human health outweigh the costs.

The studies of Haubenhofer & Kirchengast (2006-2007) and Marinelli *et al.* (2009), show a high variability of the settings used in the AAIwith regard to the therapeutic environment, the number and age of recipients and the methods of conducting the sessions. Haubenhofer & Kirchengast determined that the dogs' salivary cortisol concentrations were higher on days when there were AAIs than on a day off. In addition, cortisol levels varied with the length of sessions and the number of visits per week. Surveys done to handlers revealed that sessions between 1 and 3 hours were perceived as more intense, with fewer scheduled breaks, and that they perceived that their dogs were more physically stressed by therapeutic work than themselves. The authors then suggested that therapy dogs should be provided with several days off after participating in an AAI in order to reduce excessive arousal.

The discovery of higher cortisol levels associated with AAIs was corroborated by King *et al.* (2011) who found an increase in salivary cortisol in therapy dogs after 60 minutes of AAT performed in the hospital. Stress behaviors sampled during one minute after two hours of AAT included: panting, mydriasis, yawning, vocalizing, and licking the air. The behavior does not differ if the dogs were subjected to two minutes of stop after 60 minutes. However, increases in salivary cortisol levels are correlated with the occurrence of stressful behaviors. Fewer behavioral signs of stress were observed if dogs have two years or more of experience in AATs and/or are over 6 years of age.

Marinelli *et al.* (2009) evaluated working conditions and handlers reports on stress-related behaviours in dogs involved in AAA and AAT over a period of three years. An increase in both the frequency of the sessions and the number of participants were recorded for each dog and the handlers perceived a lower quality of the session. The age of recipients was related to the expression of stress-related behaviors, and it was seen that they were more prevalent in sessions with children under 12 years of age. The environmental conditions identified as inadequate for the dogs' welfare were represented by interference, high temperatures and lack of space.

In contrast to the results of Haubenhofer&Kirchengast (2006 - 2007) and King *et al.* (2011), no difference in salivary cortisol concentrations, comparing the days when dogs are employed in AAI and home rest days, were found by Glenk *et al.* (2013 - 2014), and Ng *et al.* (2014).

Glenk *et al*. conducted two studies to evaluate the effects of AAIs on dogs involved in activities at hospital facilities with adult recipients, analyzing salivary cortisol and behavior.In the first study dogs were employed in group sessions in psychiatric patients and salivary cortisol concentrations were found to be significantly lower in experienced dogs that were not kept on a leash and allowed to be free during sessions.No differences were found between pre- and post-session levels in experienced dogs kept on a leash and therapy dogs in training. In a later study (2014), where recipientswere people on therapy for substance abuse treatment, no significant changes in behavior were recorded over a five-week period; however, decreases in salivary cortisol were significant in the last two sessions. The authors concluded that no signs of acute stress were detectable in dogs through the two studies and that indeed they may benefit from the possibility to wander freely and interact with a group of recipients more and more familiar to them.

In the most recent study by Ines R. de Carvalho *et al.* (2019), which involved 19 dogs experienced in AAI, the concentration of salivary cortisol, the heart and respiratory rate at home (at rest) and immediately after the AAA and AAT sessions were evaluated.It was found that no significant correlation exists between the selected welfare indicators monitored at home or after sessions. The salivary cortisol concentration at home and after the session was statistically different, being higher after the session than at home. The results indicated that the period of day (morning or afternoon) had no statistically significant influence on cortisol concentration;moreover, no statistically significant difference in cortisol values was found based on sex, race, number of weekly sessions and number of recipients in sessions. In addition, the fact that the dogs were on a leash or free during the session had no effect on cortisol values after the sessions as well as the duration of the sessions.All samples collected at home had the cortisol concentration below the maximum values considered normal. In general, statistically significant differences were found between the indicators of average values at home and after sessions, with an increase in post-session values.Dogs involved in AAA had significantly higher average heart rate values after sessions when compared to those involved in AAT, although all values were still within the normal range. Dogs subjected to long journeys (> 50 minutes) to reach the setting had elevated average heart rate and respiratory rate values after the session.Most dogs had salivary cortisol concentration values within what was considered the normal range (<0.3 μg/dL) and only one dog had a very high value (1.101 μg/dL), which was attributed to the setting entry protocol and the characteristics of the session itself.Also from this study it emerged that there are no great concerns regarding the welfare of the monitored dogs, but it is important to underline the importance of avoiding some practices, such as prolonged transport (over 50 minutes) to the setting, performing interventions in too hot roomsand entering school during the children's play break.Although the salivary cortisol results of this study, regarding the number of sessions per week, are in line with the studies of other authors (McCullough *et al*., 2017), other authors have found differences (Clark *et al*., 2018) and this leaves the matteropen about how many AAI sessions per week it is advisable to carry out.

It also seems that whether the dog is on a leash or not has no effect on its welfare, contrary to what Glenk *et al.* found (2013 and 2017), which means the use of the leash should be considered on a case-by-case basis by the handler as it could be useful to control the movements of the dog while working for example with people with reduced mobility.In this study it was not possible to confirm whether familiarity with the setting affects post-intervention salivary cortisol levels (Cobb *et al.*, 2016; Ng *et al.*, 2014), heart and respiratory rate as only one in 19 dogs worked in an unfamiliar setting. Due to the fact that there is no evidence that the elevated cortisol levels in 4 dogs could be explained by any of the variables collected by the questionnaire, few comments were made that may be relevant to interpreting the responses of these 4 dogs. One of the dogs was scared by the transport by car and although the journey had lasted less than 20 minutes, they were enough to stress him significantly and this could have affected the high value of salivary cortisol. The fact that a sample was not collected after transport means that the question still remains open and that therefore further studies are needed to analyze the motivations. Two of the 4 dogs instead worked in a very warm and small environment and panted most of the time; it was not possible to measure the temperature of the room but it was also very high for the people involved in the session, as well as for the animal. This leads to the conclusion that thermal stress and playing with elderly people led to an increase in the arousal of the animal and it could explain the increases in salivary cortisol levels in these two animals; it is also suspected that one of the two dogs, more than the other was stressed by the high ambient temperature as it had a very long and thick hair. Thusthe temperature of the setting should always be measured and used as a variable in future studies because it could be related to the selected animal welfare indicators and could help to set an appropriate temperature range for animals working indoors. The last of the 4 dogs, entered the school where the activity took place during the children's break, so he was surrounded by dozens of children who wanted to interact with him; in addition, during the session he had also to interact with a restless autistic child who showed destructive behavior, so the session was interrupted several times and the dog had to crouch. These variables could explain the increase in salivary cortisol levels in this dog after the session.

Conclusion

The research carried out to date does not raise a great concern for the welfare of dogs employed as co-therapists in AAIs. Although studies report the presence of behavioral signs of stress and increases in cortisol levels during the sessions, none of the authors, based on their findings, suggest banning such practices due to severe animal welfare constraints.

One limitation of the studies carried out so far is the lack of information on causal effects and the circumstances in which the indicators of dog welfare observed vary during the AAI.For example, it would be particularly interesting, as also proposed by Glenk (2017), to investigate whether changes in dog behavior and physiological parameters occur in response to actions of the handlers or recipients or if they could be modulated by other factors. Therefore, more detailed studies on the response of co-therapist dogs to human actions would be needed.

A better understanding of how therapy dogs are influenced by environmental factors, the characteristics of AAI programs, and human management could favour the employmentof successful human-dog binomial.

Consequently, further exploration of the canine experience of AAIs should be aimed at, through the use of validated and standardised scientific instruments.

Furthermore, previous studies have focused predominantly on the short-term effects of animal participation in AAIs. Therefore, further investigations into how dogs cope with job demands in the long period would be useful.

Finally, it would be interesting to divert attention for a moment on stress assessment and orient it towards the investigation of positive welfare indicators, in order to provide information on whether and how therapy dogs can actually benefit from interaction in AAIs.

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Valutazione dello stress dei cani co-terapeuti negli interventi assistiti con gli animali: una panoramica

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Sintesi

Gli IAA, nelle loro tre forme (AAA, EAA e TAA), sono interventi finalizzati non solo a supportare ed integrare le terapie tradizionali, ma anche a sviluppare un forte legame tra animale ed uomo in contesti non terapeutici. Nonostante i benefici di queste attività sull'uomo, dimostrati da svariati studi, è importante invece non sottovalutare i rischi per gli animali coinvolti, in particolare i cani. Tali rischi sono rappresentati da diversi tipi di stress, per lo più acuti, che, se ripetuti nel tempo, potrebbero condurre, nel lungo periodo, ad una sindrome da burnout nell'animale. L'analisi della revisione della letteratura scientifica degli ultimi anni, in merito alle varie pubblicazioni sulla valutazione dello stress nel cane coinvolto negli IAA, ha confermato che non sembrano esserci grandi preoccupazioni sul benessere dei co-terapeuti se vengono evitate determinate pratiche e se si considerano i vari fattori (ambientali, umani e di gestione dei programmi) che possono influenzarlo.

Tuttavia, per il futuro sarebbe auspicabile mirare ad un'ulteriore esplorazione dell'esperienza canina nell'ambito degli IAA, attraverso l'uso di strumenti scientifici convalidati e standardizzati.