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# Hair Cortisol in Service Dogs for Veterans with Post-traumatic Stress Disorder Compared to Companion Dogs (*Canis Familiaris*)

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## ABSTRACT

Service dogs are trained to assist humans. This assistance potentially exposes them to stressors. To investigate if service dogs are exposed to more stressors than companion dogs we questioned whether hair cortisol levels differed between both groups. We studied this by cutting a tuft of hair from the neck of 19 companion and 11 service dogs. Cortisol levels were subsequently analyzed via immunoassay and compared via a simple linear regression model. The influence of coat color, season, sex, other dogs, pets, or mental health diagnoses in the household was also checked. Results showed that cortisol values did not differ between service and companion dogs. Furthermore, none of the additional variables had an influence on cortisol levels. This lead to the conclusion that the service dogs in this study did not have higher hair cortisol levels than companion dogs. Further study should be conducted as to why no difference did occur between groups and if this difference is persistent over time given that we only studied a period of up to two months' worth of hair cortisol.

## KEYWORDS

Service dog; PTSD; cortisol; hair

## Introduction

The use of service dogs is a form of Animal Assisted Intervention (AAI) in which a single specifically trained dog is deployed to continuously support the welfare of a single human handler. This handler often receives this support because he or she has a mental or physical illness or disability for which he or she requires personalized assistance to be on standby. Because this deployment potentially entails a 24/7 workload, service dogs are at risk of reduced welfare due to disturbed resting and sleeping patterns. They are additionally not in charge of their own daily schedule or social contacts and may come into contact with stressed or angry humans due to their handlers' illness or disability (Serpell, Coppinger, & Fine, 2006). Because all these factors are known sources of stress in dogs (Hubrecht, 1995; Iannuzzi & Rowan, 1991), service dogs are at risk of being exposed to stressors for extended periods of time, which can develop into the experience of chronic stress. This experience of prolonged or chronic stress not only negatively influences individual animal welfare, but can also directly influence the capacity in which a service dog is able to perform its assistance. It is therefore in the best interest of both handler and dog to keep service dogs in good welfare and to protect them from experiencing possible chronic stress.

To date, few studies have been performed specifically on the welfare and stress experience of service dogs. The welfare of related dog types within AAI has, however, been studied. A noteworthy example are therapy dogs. Several studies have questioned whether therapy dogs experience stress from assisting humans in a therapeutic setting (Clark et al., 2020; Clark, Smidt, & Bauer, 2019; Glenk

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et al., 2013, 2014; King, Watters, & Mungre, 2011; Melco, Goldman, Fine, & Peralta, 2020; Palestirini et al., 2017; Riemer, Assis, Pike, & Mills, 2016). The most common methods used to evaluate this question are a combination of behavioral assessment through structured observation and the analysis of salivary cortisol samples. From these measurements, hardly any significant differences in salivary cortisol were reported between measurements taken before and after dogs participated in a therapy session, which indicated there was no influence of assistance work on physiological stress (Clark et al., 2020; Glenk et al., 2013, 2014; van Houtert, Endenburg, Rodenburg, & Vermetten, 2021). Behavioral observations further aligned with these observations by showing low frequencies of stress related behavior in dogs during assistance work. The heart rate of some dogs was even lower after than before participation in a therapy session, indicating reduced stress and/or excitement (Clark et al., 2020).

These results suggested that dogs are capable of handling assistance work for short periods of time, though the maximum frequency of work they could perform without experiencing negative effects to their welfare remains unclear. This question was therefore asked in a study by Clark et al. (2019). In their study they observed that salivary cortisol levels were lower in dogs who assisted in multiple therapeutic sessions a week as opposed to once a week, or less than once a week. Older dogs further seemed to have lower cortisol responses than younger dogs participating in their study. Combined these results suggest that dogs have a capacity to grow accustomed to their working environment when they encounter it more frequently, and as a result mount a lower cortisol response to working situations compared to less experienced dogs. This conclusion is further supported by results of Roth, Faresjö, Theodorsson, and Jensen (2016) who observed little difference between the hair cortisol levels of companion and working (police/military) dogs over the course of three different seasons. Whether these results would also hold true for therapy or even service dogs however, remains to be seen as the longitudinal effect of assistance work in both these types of dogs has yet to be studied.

One of the ways in which the longitudinal effect of assistance work in service dogs could be explored is by following the example of Roth et al. (2016) and compare the level of hair cortisol in service dogs with that of companion dogs. Hair cortisol is a fairly new analysis method to determine physiological stress in an individual human or animal over extended periods of time. Like other free molecular structures, cortisol is built into hair by the follicles from which the hair originates. Although the exact mechanisms of this process are not yet fully understood, it is generally assumed that cortisol is incorporated during the hair's active growth phase by passively diffusing out of the surrounding plasma (Cone, 1996; Henderson, 1993; Meyer & Novak, 2012). Through this method, the concentration of cortisol incorporated in the hair is dependent on the concentrations in the plasma at the moment of incorporation. Over time hair cortisol therefore generates a registration of plasma cortisol levels, which can be retrieved through chemical analysis. There are several factors which should be taken under consideration in this process though, as not all hair cortisol can be interpreted in the same way. It is for example important to realize that hair follicles reside several millimeters below the skin surface. It therefore takes a varied period of time before the new segment of hair arrives at the skin surface and its cortisol contents can be measured (Harkey, 1993; Udo, 1978). This period depends mainly on the growth rate of the hair, which in turn may be affected by lifestyle, social interaction, month or season, sex, age, hair color, species, and the body region it is taken from (Bennett & Hayssen, 2010; Dettenborn, Tietze, Kirschbaum, & Stalder, 2012; Mesarcova, Kottferova, Skurkova, Leskova, & Kmecova, 2017; Roth et al., 2016; Terwissen, Mastro Monaco, & Murray, 2013). Hair cortisol has nonetheless been successfully linked to changes in diurnal salivary (D'Anna-Hernandez, Ross, Natvig, & Laudenslager, 2011; Papafotiou et al., 2017; Vanaelst et al., 2012), and 24-hour urinary cortisol (Russell, Koren, Rieder, & Van Uum, 2012) within an individual, which makes it a valuable tool for assessing longitudinal physiological stress experience on an individual level.

The aim of this study was to investigate whether the average hair cortisol level of service dogs is different from the average hair cortisol level of companion dogs of the same breed. Insight into this difference or the absence thereof can help determine if current service dogs experience more chronic stress than companion dogs due to their workload. This knowledge could inform service dog trainers

whether intervention is required in either the current living situation of service dogs or in the manner they are prepared for their work (LaFollette, Rodriguez, Ogata, & O’Haire, 2019).

## Material and methods

### Subjects

This study compared two groups of animals. The first group consisted of 11 service dogs for individuals with a Post-traumatic stress disorder (PTSD). The second consisted of 19 companion dogs. Both groups consisted of Labrador retriever dogs or mixed breeds which were predominantly Labrador retriever.

All PTSD service dogs had already been selected for favorable behavior prior to being deployed as a service dog. This behavior included human oriented attention seeking behavior, the ability to ignore distractions, and an even temperament. Dogs were additionally trained for at least two years in required trained behaviors, and matched to an individual with PTSD for at least half a year prior to participation in this study. For all service dogs, this process of selection, training and matching was overseen by the Dutch service dog training organization “Stichting Hulphond Nederland”, via which recruitment of service dogs was also performed. This recruitment was done as follows. Individuals with a service dog were contacted by the organization for their willingness to participate in the study. If they were willing to participate, the organization would bring them into contact with a researcher who would make an appointment to explain the steps necessary to participate. Only after having been provided with this information both verbally, and in writing participation became possible to ensure informed consent of all owners/handlers before their dog participated.

All companion dogs were privately owned dogs who were volunteered by their owners for participation. Recruitment for this group was performed via social media platforms or through personal communication. Like with the service dog group, owners could only volunteer their dog for participation after receiving verbal instructions by a researcher. Again this was to ensure informed consent of all owners/handlers before their dog participated in the study. Further details on both groups can be found in Table 1.

### Measurements

A tuft of 2-centimeter-long hair was collected from each dog for cortisol analysis. Samples were retrieved from the base of the back of the neck of each dog by their owner (Meinders, 2017). Collection of samples was done by cutting the hair as close to the skin as possible without damaging the skin. This method was chosen because the use of sheering tools can cause stress in dogs who are not familiar with the sound. The desired weight of the hair sample (when trimmed down to two centimeters from the root) was 40 mg or more (a tuft of roughly 2 fingers wide). Collected hair samples were initially wrapped in aluminum foil to protect them from sunlight and stored at room temperature in a dark environment. Subsequent extraction of cortisol was performed based on the protocol by Davenport, Tiefenbacher, Lutz, Novak, and Meyer (2006). In summary this meant that

**Table 1.** Age (n = 11, n = 16), sex (n = 11, n = 16) and coat color (n = 11, n = 19) of both the service dogs and companion dogs who participated in this study. Some percentages do not add up to 100% due to rounding. Additionally the n of the companion dog group differs between variables due to missing data.

|                | Age            |                   | Sex       |             | Coat color |            |            |
|----------------|----------------|-------------------|-----------|-------------|------------|------------|------------|
|                | $\mu$<br>years | $\pm$ Sd<br>years | Male<br>% | Female<br>% | Black<br>% | Blond<br>% | Brown<br>% |
| Service dogs   | 3.27           | 2.05              | 82        | 18          | 45         | 18         | 36         |
| Companion dogs | 5.38           | 3.56              | 31        | 69          | 32         | 26         | 42         |
| All dogs       | 4.52           | 3.17              | 52        | 48          | 37         | 23         | 40         |

samples were washed twice with isopropanol to remove any external corticosteroids that could interfere with analysis. Samples were subsequently dried and reduced to powder. Thirty mili gram ( $\pm 5$  mg) of this hair powder was dissolved in 1.5 mL methanol to extract steroid hormones. After 24 hours the remaining powder pallet was removed from the methanol. The methanol itself was then evaporated to leave only the steroid residue behind. Analysis of this steroid residue for cortisol was finally performed via a Salimetrics High Sensitivity Salivary Cortisol Enzyme Immunoassay (Salimetrics, 1–3002). The average intra-assay variation was 2.4%.

Specific details on each dog's sex, and age, and living conditions were additionally recorded through a questionnaire filled out by their owner or handler. This included information on the number of hours each dog walked per day, the presence of other dogs in the household, the presence of other pets besides dogs in the household, and the occurrence of mental health diagnosis in humans (both adults and children) of the household the dog lived in. Specifics regarding a dog's coat color and the month in which the sample was collected were registered by a researcher upon receiving the hair sample and questionnaire from the dog owner (Tables 1, 2, and 3).

### **Statistical analysis**

Statistical analysis was performed in R version 4.0.3 with R studios (R Core Team, 2018). A total of eight variables were analyzed for their difference between two participant groups. These variables were PTSD service dog/companion dog, coat type (light = blond, dark = brown/black), month of sample collection, dog sex, dog age, the presence of other dogs in the household, the presence of other pets besides dogs in the household, and the presence of mental health diagnosis in humans living in the household. Normality of collected data were judged via a QQ plot and equality of variances was judged via Bartlett test of homogeneity of variances. Analysis of the dataset itself was subsequently performed via a student's T-tests, simple/multiple linear regression between sets of two variables. Simple linear regressions were applied to the variables: PTSD service dog/companion dog, dog sex, dog age, coat type, the presence of other dogs in the household, the presence of other pets besides dogs in the household, and the presence of mental health diagnoses in humans living in the household. Categorical variables were first transformed to dummy variables to make analysis via linear regression possible. This was also true for the variable month of sample collection' which was analyzed via multiple regression.

### **Ethical statement**

Ethical advice regarding the procedures performed in dogs in this study was sought from Utrecht University's Animal Welfare Body. This Body can be approached with the question whether the experimental animal work requires ethical approval. Because no invasive measurements were taken however the Animal Welfare Body communicated that the procedures performed were not considered an animal experiment and that the protocol of this study did not require further ethical approval (decision taken on November 27 2017).

Ethical review and approval of the questionnaire used in this study was obtained from the medical ethical committee of the Utrecht Medical Center, Utrecht, The Netherlands. This approval was part of a larger study to the interaction between service dogs and military veterans which is covert in dossier NL64117.041.18. The procedure to acquire data additionally included informed consent of the participating veterans.

Results

Linear regression

The mean hair cortisol found in service dogs was 9.69 pg/mg hair ( $\pm 2.77$ ) while that of companion dogs was 8.65 pg/mg ( $\pm 3.09$ ). To check if these means differed from one another, a simple linear regression (hair cortisol =  $\beta_1 \times$  “ PSD/companion dog status” +  $\beta_0$ ) was calculated to predict hair cortisol level based on PSD/companion dog status. With this calculation a non-significant regression equation was found ( $p = 0.37$ ,  $\beta_1 = 1.04$ ), with an  $R^2$  of 0.03.

Additional analyses via linear regression were performed to check if sex, age, month of collection, coat type, hours walked daily, the presence of other dogs, the presence of other animals, and/or the presence mental health diagnoses in the household had an influence on the hair cortisol samples retrieved during this study. None of these variables showed a significant relationship to the retrieved levels of hair cortisol (Table 4)

Dataset description

The retrieved sample weight was below 40 mg in 7 out of 30 hair samples. This required the use of adjusted dilution volumes during analysis for samples with low weight. Because of this correction no hair cortisol values were missing from the dataset. Some descriptive information of samples was missing though, since the age and sex of three companion dogs was not registered.

When comparing characteristics in both groups of dogs via students t-test, there was no significant difference between the coat type (black/brown or blond;  $p = 0.63$ ), the presence of other dogs ( $p = 0.72$ ), or presence of other animals in the household ( $p = 0.92$ ). A difference was present, however, between the sex ratios of both groups ( $p = 0.01$ ), the months in which the hair was retrieved ( $p < 0.01$ ), the hours walked daily ( $p = 0.05$ ), and in the presence of mental health diagnoses in humans of the household ( $p < 0.01$ ). Regarding the sex ratios, the service dog group contained a higher percentage of male (82% vs 31%) and a lower percentage of female (18% vs 69%) dogs when compared to the companion dog group. Regarding months, samples in service dogs were taken predominantly in winter months while samples in companion dogs were taken predominantly in spring or summer months. Regarding hours walked daily, results showed that service dogs walked more hours with an average of 2.36 vs 1.62 in companion dogs. Regarding the presence of mental health diagnoses results showed that service dog households had more diagnoses than those of companion dogs, with 2.27 versus 0.32 reported diagnoses per household (see also Table 2). Regarding the average age of the dogs in both groups, no significant difference were found. This was likely caused by the presence of some older dogs in the companion dog group who skewed the age distribution of this group, while the service dogs were more of similar age.

**Table 2.** Hours walked (per day), percentage of households with other dogs or other pets present, and absence or presence of humans with psychological deviations (number of deviations present per household) of the service ( $n = 11$ ) and companion dog ( $n = 19$ ) groups.

|                | Hours walked<br>$\mu \pm Sd$ | Other dogs present<br>% | Other pets present<br>% | Mental health diagnoses<br>$\mu \pm Sd$ |
|----------------|------------------------------|-------------------------|-------------------------|-----------------------------------------|
| Service dogs   | 2.36 $\pm$ 1.12              | 45                      | 55                      | 2.27 $\pm$ 1.42                         |
| Companion dogs | 1.62 $\pm$ 0.83              | 53                      | 58                      | 0.32 $\pm$ 0.58                         |
| All dogs       | 1.89 $\pm$ 1.00              | 50                      | 57                      | 1.03 $\pm$ 1.35                         |

**Table 3.** The month in which the hair of service and companion dogs was collected ( $n = 11$ ,  $n = 19$ ).

|                | Jan | Mar | Apr | May | Jun | Sep | Okt | Dec |
|----------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Service dogs   | 2   | 0   | 1   | 0   | 2   | 1   | 3   | 2   |
| Companion dogs | 0   | 9   | 4   | 3   | 3   | 0   | 0   | 0   |
| All dogs       | 2   | 9   | 5   | 3   | 5   | 1   | 3   | 2   |



**Table 4.** The p value, r2, Beta, and intercept values of simple linear regressions between various independent variables and the hair cortisol concentration in the various dogs within this study.

| Independent variable compared to hair cortisol | p    | R2   | β1        | β0    |
|------------------------------------------------|------|------|-----------|-------|
| PSD/companion dog status                       | 0.37 | 0.03 | 1.04      | 8.65  |
| Sex                                            | 0.40 | 0.03 | −1.03     | 9.59  |
| Age                                            | 0.58 | 0.01 | −0.11     | 9.56  |
| Month of collection                            | 0.17 | 0.05 |           | 9.28  |
|                                                |      |      | March     | −0.38 |
|                                                |      |      | April     | 0.37  |
|                                                |      |      | May       | −1.72 |
|                                                |      |      | June      | 0.01  |
|                                                |      |      | September | 1.63  |
|                                                |      |      | October   | −0.95 |
|                                                |      |      | December  | −0.30 |
| Coat type                                      | 0.47 | 0.02 | −0.96     | 9.77  |
| Hours walked daily                             | 0.83 | 0.00 | 0.11      | 8.81  |
| Presence of other dogs                         | 0.95 | 0.00 | 0.07      | 8.99  |
| Presence of other pets                         | 0.10 | 0.09 | 1.81      | 8.07  |
| Mental health diagnoses                        | 0.16 | 0.01 | −0.17     | 9.37  |

Note: Reference variables for statistical analysis were as follows: Companion dog (Status), Female (Sex), Januari (Month), Light (Coat type), No other dogs present (Other dog presence), No other pets present (Pet presence). Age, Hours walked daily, and Mental health diagnoses were numeric variables.

Analysis of equal variance was performed via Bartlett test of homogeneity of variances in R ( $p = 0.66$ ).

**Discussion**

This study investigated if the average hair cortisol level of service dogs differed from that of companion dogs of the same breed. Our results showed no difference in hair cortisol based on this division. This observation leads to the conclusion that service dogs do not appear to experience more physiological stress over time than companion dogs do. Nonetheless it is possible that cortisol levels were influenced by variables other than the division between service dogs and companion dogs. Because of this possibility the influence of several variables on the results found was checked.

**Influence of dog-specific variables**

A variable, which possibly influences the level of retrieved hair cortisol independent of the experience of physiological stress, is a dog’s coat type. A study by Bennett and Hayssen (2010) for example found dark hair or dark portions of hair to contain less cortisol than light portions of hair in German Shepherd dogs. This is contrary to results found in our study as this study did not find an influence of coat type on the overall retrieved level of hair cortisol in Labrador retriever dogs. A possible explanation for this difference in results is that the difference between light and dark colored hairs found by Bennett and Hayssen (2010) was not entirely based on coat type but rather on an underlying confounding factor like breed (Mesarcova et al., 2017). This theory is supported by results found by Nicholson and Meredith (2015) and Rosén (2016), who also found no differences in hair cortisol based on coat type in several dog breeds other than German Shepherds (Border collies, Jack Russell terrier, Labrador retriever, Cavalier King Charles spaniel, Shih Tzu and Springer spaniel).

Age is a second variable that can potentially influence the level of hair cortisol in an animal independent of physiological stress. Although the mean age of service dogs and companion dogs did not differ significantly from one another in our study, the average age of companion dogs tended to be higher than that of service dogs. This difference could therefore have influenced found cortisol levels in retrieved hair, since some assume that cortisol levels in dogs’ coats increase with age (Mesarcova et al., 2017). According to Mesarcova et al. (2017) however, this



difference has not yet been confirmed. Indications additionally exist that progressing age lowers instead of raises cortisol levels in dogs. In their study Clark et al. (2019) for example, found that older therapy dogs had lower salivary cortisol levels in response to therapy work than younger conspecifics. Given that, like hair cortisol (Cone, 1996; Henderson, 1993; Meyer & Novak, 2012), salivary cortisol is linked to free cortisol levels in the blood of an individual (Aardal & Holm, 1995; Beerda, Schilder, Janssen, & Mol, 1996), this makes it unlikely that hair cortisol levels would rise while salivary cortisol decreased with age. This does not rule out the possibility of hair cortisol rising with age necessarily however, since the lowering of cortisol seen in dogs during the study of Clark et al. (2019) might have been influenced by the experience dogs had with the situation they were put in (therapy session). Regardless, this study did not find any influence of age on hair cortisol levels retrieved during our study, which is in line with findings of Mesarcova et al. (2017).

A third and final dog-specific variable which could have influenced the level of hair cortisol found in our study, is sex. Although it is possible that differences in hair cortisol level based on sex or spay/neuter exist in mammals, such a difference has not yet been found in dogs (Bennett & Hayssen, 2010; Mesarcova et al., 2017; Svendsen & Sondergaard, 2014). Results from our study are in line with these findings, as this study did not find a relationship between sex and hair cortisol despite there being a significant difference in the sex ratio of the PSD and companion dog groups (82% vs 31% male).

### ***Influence of living conditions***

Besides dog-specific variables there are also several variables regarding a dog's living conditions which might influence the accumulation of hair cortisol. This study therefore checked for the influence of several potentially stress increasing or stress reducing variables. Regarding stress reduction, this study checked for the influence of the total hours a dog walked per day and the presence of other dogs in the household. This was done because both social and spatial restriction are known to negatively affect welfare in dogs (Beerda, Schilder, Van Hooff, De Vries, & Mol, 1999). Walking more and having free contact with conspecifics could therefore theoretically reduce the experience of chronic stress in dogs and by extension the total amount of cortisol found in hair. Although the service dogs in our study walked more hours per day than the companion dogs, no relation between these variables and hair cortisol was found. Possibly this is due to the environment in which dogs walked. In humans it has been found that walking in a forest or natural area reduces cortisol levels, while walking in an urban area does not (Kobayashi et al., 2019). Because service dogs frequently accompany their handlers to stores, in public transport and in other urban area's when going for walks it is therefore possible that no effect of walking on cortisol levels is present due to the walking environment. It is further possible that walking increased instead of decreased the level of found cortisol in service dogs since cortisol is known in some mammals to increase with increased activity (de Groot, de Jong, Prelle, & Koolhaas, 2000; de Jong et al., 2000) therefore canceling out any reduction effects.

Additionally no relationship between the presence of other dogs in a household and retrieved hair cortisol levels was found. Though it is possible that the presence of another dog helps to prevent stress from social isolation in dogs, it is also possible that it simultaneously increases cortisol levels. Since the presence of another dog increases the opportunity to show play behavior, cortisol could be elevated in dogs who live with a conspecific. It is additionally possible for cortisol to increase in the presence of another dog if the interactions between both animals are hostile or competitive. Since no difference was found between living with and without a conspecific though, neither of the three theories can be excluded or proven in our study.

Regarding stress induction this study further checked for the influence of pets other than dogs in the household and the presence of mental health diagnoses in humans of the household. As expected the presence of mental health diagnoses in humans of the household was significantly higher for service dogs than for companion dogs. Mostly this was due to the presence of PTSD in service dog

owners/handlers, though issues in other individuals like children were also reported. This had no detectable effect on hair cortisol levels in the service dogs however. Dogs who lived with other pets meanwhile appeared to have higher hair cortisol levels than those who lived alone or only with other dogs. This effect was not significant though. The pets most frequently reported were cats, which could lead to heightened stress in dogs if both species cannot peacefully live side by side in a restricted environment or have to compete for human attention. The presence of some rodents (Guinea pigs, gerbils, hamsters) or rabbits was additionally reported which might also cause stress elevation in dogs due not to being allowed to perform prey directed behavior. The presence of livestock (horses, sheep, chickens) was reported for some dogs, though these animals did not live in the same environment as the dogs (indoor vs outdoor housing) and might therefore have had limited influence on stress experience. Overall the dogs' reaction to these various animal species may therefore have been diverse which would explain why no significant relation was found between their presence and cortisol levels. Future studies should therefore consider less categorical variables to allow for a wider range of potential stressors and interactions between them to be studied.

### ***Influence of environmental variables***

Besides dog-specific variables and living conditions there was an environmental variable which might have influenced retrieved levels of hair cortisol during this study. This variable is the month in which hair was collected for each dog. In our study most service dogs were sampled in the autumn or winter months while most companion dogs were sampled in spring months (based on animal availability). A study by Roth et al. (2016) found a seasonal effect on hair cortisol in dogs, with cortisol being higher in January than it was in either September and May. This study did not find an effect of season on hair cortisol, which is in line with the results of a study by Thun, Eggenberger, and Zerobin (1990) who also did not find this effect on plasma cortisol levels in dogs. An explanation for the results found by Roth et al. (2016) could therefore be that hair cortisol levels in dogs in autumn and winter months are affected by external factors like the onset of cold weather or the shortening of photoperiod in the months leading up to January. Since the study by Roth et al. (2016) was conducted in Sweden while the study by Thun et al. (1990) and this study were conducted in the milder climates of Switzerland and the Netherlands respectively, an influence of climate and length of daylight in winter months on coat development in dogs cannot be excluded.

### ***Limitations and future research***

Because of all the above we can assume that the results found in this study were not influenced by the discussed factors. Nonetheless the results presented in this study know several limitations. The most prominent of these limitations are the limited sample size of this study and that we only observed hair cortisol over two months. Differences between groups of dogs may be bigger if measurements are taken in larger sample sizes and over longer periods of time, which may lead to different conclusions than the ones drawn in this study. Future research should therefore focus on larger studies over longer periods of time to determine whether or not the results of this study are relevant to a wide group of service dogs.

Additionally it can be questioned if symptom severity of those afflicted with mental health diagnoses in humans might have influenced results. In our study only the presence or absence of mental health diagnoses in humans was used as a potential stressor for (service) dogs. Severity could vary between individual humans though which in turn could affect dogs differently. It is therefore recommended that more study is performed to the influence of different mental health diagnoses in humans on dog welfare.

It could also be discussed whether hair cortisol is a good medium to measure differences in experienced stress between dogs. No clear baseline for hair cortisol in stress free dogs is currently

known, which left us with the option of comparative research between groups. We chose to compare service dogs with companion dogs because these dogs resemble the most common form of housing and care in dogs. Nonetheless it cannot be excluded that companion dogs experience chronic stress due to the way they are kept by humans. Future research to the presence of stress in both companion and service dogs should therefore consider how to account for this knowledge gap by for example combining the measurement of hair cortisol with other measurements like heart rate monitoring and behavioral observations.

Finally additional measurements should be conducted in future research to compare the workload, working hours, or number of active hours of various types of dogs in relation to their experience of stress. The frequency at which dogs are exposed to stressors has not been addressed in this study which instead focussed more on categorical presence.

## Conclusion

In conclusion there was no difference in hair cortisol between the group of service dogs and the group of companion dogs observed during this study. Additionally, no significant influence of several other variables was found on this result. This lack of difference in cortisol levels suggests that the service dogs who participated in this study did not experience more physiological stress over time than the companion dogs. An explanation for this favorable result can be the high quality of the training program of the service dogs who participated in this study. These dogs underwent an extensive 2-year training with a specialized service dog organization, were selected for suitable behaviors, were matched with a suitable handler, and were monitored on a half year basis post placement with their handler. Whether or not the conclusion of this study therefore holds true for dogs trained by other organizations and other types of dogs in AAI (like dogs for autism, physical disabilities, diabetes) deserves further study.

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## Data Availability Statement

The data presented in this study are available on request from the corresponding author.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Institutional review board statement

Ethical advice regarding the procedures performed in dogs in this study was sought from Utrecht University's Animal Welfare Body. This Body can be approached with the question whether the experimental animal work requires ethical approval. Because no invasive measurements were taken however the Animal Welfare Body communicated that the procedures performed were not considered an animal experiment and that the protocol of this study did not require further ethical approval (decision taken on November 27 2017).

Ethical review and approval of the questionnaire used in this study was obtained from the medical ethical committee of the Utrecht Medical Centre, Utrecht, The Netherlands. This approval was part of a larger study to the interaction between service dogs and military veterans which is covert in dossier NL64117.041.18. The procedure to acquire data additionally included informed consent of the participating veterans.

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