EFFECTS OF POSITIVE CARETAKER INTERACTIONS ON DOG WELFARE IN COMMERCIAL BREEDING KENNELS

by

JoAnna C. Rogowski

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STATEMENT OF COMMITTEE APPROVAL

Dr. Candace C. Croney, Chair

Department of Comparative Pathobiology/Animal Sciences

Dr. Alan M. Beck

Department of Comparative Pathobiology

Dr. Janice E. Kritchevsky

Department of Veterinary Clinical Sciences

Approved by:

Dr. Sanjeev K. Narayanan

To Grandpa Gene.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
CB	Commercial Breeding
sIgA	Secretory Immunoglobulin A
HCC	Hair Cortisol Concentration
CI	2-minute Caretaker Interaction
TO	Treat-Only Interaction
NAIA	National Animal Interest Alliance
USDA	United States Department of Agriculture
NS	Nervous System
SNS	Sympathetic Nervous System
HRV	Heart Rate Variability
HPA-axis	Hypothalamic-Pituitary-Adrenal-axis
BCS	Body Condition Score
CBE	Commercial Breeding Establishment
C-BARQ	Canine Behavioral Assessment and Research Questionnaire
HAIs	Human-Animal Interactions
SST	Strange Situation Test
FIDO	Field Instantaneous Dog Observation Tool
RYG	Red, Yellow, Green Approach Test
FIDO+	Extended Field Instantaneous Dog Observation Tool
RYG+	Extended Red, Yellow, Green Approach Test
GLME	Generalized Linear Mixed Effects Model

ABSTRACT

A large portion of the demand for purebred dogs in the United States is met by commercial breeding (CB). CB is a contentious issue, and concern exists surrounding the quality and quantity of human-animal interactions in CB kennels. Quality of caretaker interactions has been demonstrated to affect welfare in livestock and laboratory animals, yet is widely understudied in kenneled dogs, especially those kept for CB. It therefore warrants investigation. Thus, the aim of this study was to determine the effect of a short, regular, positive caretaker interaction on physiological and behavioral metrics of dog welfare in CB kennels. Adult bitches (n = 47) from two CB kennels received a daily interaction with a familiar caretaker for two weeks. Half of the dogs (n = 24) received a 2-minute caretaker interaction with treats (CI), and the other half (n = 23) received treats only (TO). All other human interactions were limited to routine husbandry. Fecal secretory immunoglobulin A (sIgA), hair cortisol concentration (HCC), and behavior in response to human approach were measured at baseline (Day 0), after two weeks of treatment (Day 14), and two weeks after treatment ended (Day 28). Behavior during treatment delivery was scored from video on days 1, 2, 8, 9, 13, and 14. General linear mixed models were used with treatment type and timepoint as fixed effects, dog nested within pen as random effects, and welfare metrics as dependent variables. Data from both facilities were analyzed and presented separately. In Facility 1 (n = 25), treatment type did not affect hair cortisol concentration (HCC) or fecal secretory immunoglobulin A (sIgA). However, HCC increased significantly from Day 0 to Day 28 ($X^2 = 5.83$, $p = 0.016$) and fecal sIgA decreased significantly ($X^2 = 21.52$, $p < 0.001$) over all three timepoints. Affiliative behavior in response to human approach increased over time with no significant effect of treatment type or tester (X^2=10.549, p=0.001). Additionally, time spent in proximity to the caretaker significantly increased in both treatment groups during the daily interaction (CI: X^2=14.047, p<0.001, and TO: X^2=5.121, p=0.024). In Facility 2 (n = 22), there was no effect of treatment type on physiological metrics, however, HCC decreased in time ($X^2 = 6.66$, $p = 0.009$) in both treatment groups combined. Affiliative response to human approach increased over time in Facility 2 (X^2=13.5782, p=0.001). During daily interactions, dogs from the TO group displayed increased affiliative ($X^2 = 8.58$, $p = 0.003$) and decreased ambivalent ($X^2 = 10.42$, $p = 0.001$) behaviors over time, while dogs from the CI group showed increasing latency to

approach the caretaker ($X^2 = 4.38$, $p = 0.033$). Changes in physiological and behavioral metrics differed by facility and treatment group. Factors such as variation in treatment quality and prior caretaker-animal relationship may play a role in dogs' responses to the treatment. These results suggest that a caretaker interaction has the potential to improve welfare in dogs residing in CB kennels. However, careful consideration must be taken when implementing new protocols to avoid unintended increases in stress. For some adult dogs unaccustomed to extended, structured interactions with their caretakers, a 2-minute session may have resulted in increased physiological and behavioral stress, suggesting that a longer interaction might have jeopardized rather than improved their welfare. For these dogs, a more gradual introduction to human interactions may be more beneficial. This study offers new insight on the implementation of socialization, counterconditioning, and caretaker-dog interaction practices to maximize positive welfare in CB kennels. Future research is needed to further validate and expand upon these findings.

CHAPTER 1. INTRODUCTION

In the United States, many people share strong bonds with dogs. As of 2022, 69 million households in the US reported owning at least one dog (APPA, 2022), which has increased from the reported 46.3 million in 2012 (APPA, 2012). Dog ownership has been associated with numerous benefits, including lower blood pressure (see Kramer et al., 2019; Arhant-Sudir et al., 2011 for reviews), higher levels of physical activity (e.g., Cutt et al., 2007), lower levels of anxiety (e.g., Hinic et al., 2019;), and lower levels of loneliness (e.g., Powell et al., 2019) in dog owners versus those who do not keep dogs. Dog owners have been reported to have an easier time forming new relationships (Wood et al., 2015; Gueguen & Ciccotti, 2015) and reportedly share closer bonds with human counterparts (Sheppard, 2019). Along with physical and mental health benefits, many people enjoy close bonds with dogs. A great number of dog owners consider their dogs to be members of their family. One survey reported 57% of respondents holding this view (Bir et al., 2016). Considering these factors, it is not surprising that the demand for dogs continues to increase. In fact, roughly 8.3 million dogs are required each year to meet demand (Cushing, 2020). However, throughout the COVID-19 pandemic, demand for dogs has increased a great deal more than was predicted (Morgan et al., 2020). Animal shelters across the country have reported very high dog adoption rates (Smith, 2020). This dramatic increase may be explained by the fact that during a time when the public was advised against social interaction, many people sought companionship in dogs (Vincent et al., 2020; Juneau, 2021; Gajanan, 2020), and those who acquired a dog during the pandemic reported improved physical and mental well-being (Juneau, 2021). High demand for dogs, exacerbated by the COVID-19 pandemic raised questions about how that demand is met. Commodification of dogs is necessary, yet with it comes societal concerns about how dogs are raised and cared for. Because of the strong bond people share with dogs, there are ethical concerns about dog welfare and scrutiny over their sourcing.

In order to address public concerns, the question of how dogs are made available to the public must be answered. One survey in the United States asked dog-owning respondents how they acquired their dog. While nearly 40% reported adopting from a shelter or rescue organization, over 50% of respondents indicated that they had obtained their dog either from a breeder or retailer (Bir et al., 2016). Other studies examining the percentage of dogs in the United States that are purebred found similar numbers: 56% (American Pet Products Association) and 48.7% (American Veterinary Medical Association, 2018) (as cited by Humane Society of the United States, 2021. This high demand for purebred dogs warrants investigation into their supply to the public. There are many reasons for which people may desire purebred dogs. Some acquire a dog for a specific purpose (e.g., herding, guarding, or competition) and seek out a dog bred to have traits that will aid in that purpose. Others may believe that a purebred dog can offer higher predictability in terms of health, physical appearance, and behavioral characteristics (Bauer & Croney, 2018). Whatever the reason, a large percentage of the demand for dogs is being met by purebred dogs, and it could be assumed that without purebred dogs, it would not be possible to meet that demand. Many activist organizations imply that shelters and rescues can supply purebred dogs to those who seek them via rehoming, citing that approximately 25% of dogs in shelters are purebred (e.g., Cvetkovska, 2022; Davidson, 2020; HSUS, 2021a). It is difficult to ascertain true statistics regarding the kinds of dogs available in US shelters due to the lack of governing body or oversight of these organizations (NAIA 2015, HSUS, 2021). However, a study done by the National Animal Interest Alliance (NAIA) found that the proportion of purebred dogs in US animal shelters is closer to five percent (NAIA, 2015). In this study, 18 shelters across the country were monitored weekly for one year and reported the proportion of purebred dogs available for adoption (NAIA, 2015). Limitations aside, the difference between the results of this study and those reported by many activist groups is staggering. Additionally, authors found that removing Chihuahuas and "Pit Bull"-type dogs due to their overrepresentation lowered that percentage from five to three percent (NAIA, 2015). Regardless, it is clear that shelters alone cannot meet the demand for purebred dogs, therefore, they must be supplied in some form.

There are many ways in which dogs are made available to the public, including through pet stores, hobby breeders, commercial breeders, non-regulated breeders, breed-specific rescue groups, and animal shelters. As the supply of dogs is a highly contentious issue, many activist organizations hold and promote strong opinions as to how dogs should be sourced. One possible source of these opinions is the portrayal of graphic images of seemingly unhealthy or injured dogs living in squalid conditions by the media, calling on the public to avoid dogs from 'puppy mills'. While there is no established definition of the term, 'puppy mill,' it is clear that in these establishments profit takes precedence over welfare. However, it is important to note that 'puppy mills' differ from commercial breeding (CB) kennels. CB kennels operate legally by meeting welfare standards set by the United States Department of Agriculture (USDA) and their state of operation. Licensed facilities are also subject to unannounced inspections (USDA, 2016). Unfortunately, the distinction between the two terms is not well-known, and because of this, CB kennels are often included in this public scrutiny (Croney, 2019). Therefore, many are concerned about how well the physical and mental needs of dogs in these facilities can be met (Croney, 2019).

Because CB kennels have been lumped in with other large-scale and sometimes unregulated breeding operations in the public eye, puppies originating in CB kennels are often believed to have many physical and behavioral health issues (McMillan, 2017; McMillan et al., 2011). Another concern is the frequency and quality of the interactions these dogs have with their caretakers (Croney, 2019). As puppies born in these facilities experience a large part of their critical period of socialization in the kennel, it is possible that these puppies may grow up to be fearful of certain stimuli if they are not properly socialized. Since the caretaker is the primary source of human interactions while dogs reside in kennels, these dogs may generalize the quality of those interactions to experiences they may have with other people.

The quality of human-animal interactions and its effect on animal welfare has long been studied in livestock. A large number of studies have found human-animal interactions of poor quality to result in both avoidance of humans and poor welfare in chickens (e.g., Cransberg et al., 2000), cattle (e.g., Rushen et al., 1999), and swine (e.g., Hemsworth et al., 1981). In dogs, aversive training methods have been found to negatively impact the dog-human relationship (e.g., Hiby et al., 2004; Deldalle et al., 2014; see also: review by Ziv, 2017). Additionally, there is concern surrounding the fate of dogs at the end of their breeding careers. Some fear these dogs may face convenience euthanasia (Croney, 2019), or inhumane killing. Finally, many feel that the breeding of purebred dogs exacerbates perceived problems of dog overpopulation. Because of the approximately 3.1 million unwanted dogs entering animal shelters each year (ASPCA, 2022), many activists place blame on those breeding purebred dogs, stating that they are "flooding the market" by making dogs with no guaranteed homes available to the public. However, the majority of homeless dogs are mixed breed (NAIA, 2015), which creates a mismatch between the kind of dogs available in shelters and the demand that exists for purebred dogs. While the euthanasia of unwanted dogs is a sad reality, solely blaming dog breeders is misguided. Further, those breeding purebred dogs are simply responding to consumer demand (Fennell, 1999; Croney, 2019). However, due to this and the other aforementioned public concerns, bans on sales of puppies in pet stores are increasing. While these may help to ease public concern, they create opportunities for unregulated, possibly sub-standard breeders to fill the gap, making it even more important to study and ensure the welfare of dogs in commercial breeding kennels. It is clear that CB kennels are an important source of a regulated supply of dogs to the public. However, the current scarcity of research done in this area, along with the ethical obligation to care for dogs with which humans share a close bond, warrants further investigation into the welfare of dogs bred and raised in these facilities.

CHAPTER 2. LITERATURE REVIEW

2.1 Conceptions of Animal Welfare

Animal welfare refers to an animal's physical and mental state relative to its attempts to cope with the conditions in which it lives (World Organization for Animal Health [OIE], 2019; Broom, 1986). The establishment of this definition has taken many decades of scientific debate. Many credit Ruth Harrison's book, *Animal Machines,* which spoke about the treatment of livestock as inanimate machines, sparking concern for the welfare of farm animals (Broom, 2014). Shortly after the release of *Animal Machines*, the Brambell committee was developed and published in the Brambell Report (1965). This report mandated that animals should have the freedom to "stand up, lie down, turn around, groom themselves, and stretch their limbs" (Brambell et al., 1965). This led to the creation of the Farm Animal Welfare Council, which published the "Five Freedoms": (1) freedom from hunger and thirst, (2) freedom from discomfort, (3) freedom from pain, injury, or disease, (4) freedom from fear and distress, and (5) freedom to express normal behavior (McCulloch, 2013). The "Five Freedoms" can be accompanied by the "Five Provisions": (1) provide access to fresh water and a healthy diet, (2) provide appropriate shelter and a comfortable resting area, (3) provide prevention from, and rapid diagnosis and treatment for, pain, injury, and disease, (4) ensure treatment and conditions which avoid mental suffering, and (5) provide sufficient space and access to conspecifics (Webster, 2005).

In an attempt to account for the complexity of animal welfare, Fraser and colleagues (1997) developed what is commonly known as the "Three Circles Model" (Fraser et al., 1997). The model shows three overlapping circles: basic health and functioning, natural living, and affective states. The basic health and functioning circle entails meeting the most basic needs of the animal, such as food, shelter, and veterinary care. The natural living circle surrounds the freedom to express normal, species-specific behavior and an environment in which that is supported. Finally, the affective states circle seeks to provide the animal with a positive mental state, with the term "affective states" referring to the animal's evaluation (hedonistic or aversive) of its experiences (Fraser et al., 1997; Yeates & Main, 2008).

Along with the "Three Circles Model", the "Five Domains model" was developed (Mellor & Reid, 1994) and adapted to include positive affect and consideration of the animal's mental state

(Mellor & Beausoleil, 2015). The model includes four physical and functional domains, which are broken down into survival-related (nutrition, environment, and health), and situation-related (behavior) factors. The fifth domain, affective experience, provides examples of mental state for each of the physical/function domains.

The "Quality of Life (QoL)" framework serves as a holistic approach to the complicated concept of animal welfare, (Yeates, 2011). While there is no widely accepted definition of QoL (Green & Mellor, 2011; Taylor & Mills, 2007a) it takes into account both negative and positive affective states, what is "normal", individual versus group assessment of welfare, and change over time (Morton, 2007). All the above-mentioned models of animal welfare provide guidelines for and examples of good welfare. However, no one model encompasses animal welfare in its entirety, making it important to understand the commonalities and benefits captured by each model.
While the models differ in key areas, what each reflects is the underlying notion that as animals carry a certain level of intrinsic value, whether their use provides food, labor, or companionship, their interests are of moral concern (Fraser et al., 1997). Positive welfare is not always achieved simply by the absence of negative affective states. Animals must also be able to express natural behaviors, avoid negative affective states as much as possible, and have an overall positive quality of life. In order to ensure positive welfare, one must be able to scientifically assess the welfare state of an animal.

2.2 Measuring Animal Welfare

Any theoretical conception of animal welfare must be critically considered, as welfare is intimately linked with its empirical assessment (McCulloch, 2013). Animals must be able to sufficiently cope with their environments in order to maintain homeostasis (McEwen & Wingfield, 2010). Certain internal or external stimuli can lead to the loss of homeostasis, which in turn will cause the animal to respond either internally (i.e., physiological mechanisms) or externally (i.e., behavioral mechanisms) (Broom & Johnson, 1993). Prolonged exposure to stimuli which cause imbalances, or inability to maintain homeostasis often leads to stress (Saplosky, 2004), which jeopardizes welfare. It is important to distinguish the two types of stress that occur: eustress and distress. Eustress is positive stress and can have similar effects to distress, like increased heart rate. However, these effects are derived from positive experiences, such as play or copulation (Mills et al., 2010; Colborn et al., 1991). Alternatively, distress is negative stress, which can come from short-term experiences such as pain from an injury, or long-term failure to cope with the environment (Mills et al., 2010). For the remainder of this text, the term "stress" will refer to negative stress, or distress.

There are many tools available to measure animal welfare. Metrics can be broken down into three categories: physical, physiological, and behavioral. Physical welfare metrics can include the presence or absence of injury and illness, assessment of body condition and reproductive success, and examination of the environment in which an animal lives. Physiological metrics examine the functioning of the neural and endocrine system. Examples include heart rate or heart rate variability, immune function, and hormone levels (i.e., cortisol). Finally, behavioral metrics can include presence or absence of abnormal behavior, evaluation of time budgets, and response to human approach. When assessing welfare, it is important to utilize multiple metrics, as examining only one measure may not lead to an accurate assessment. It is important to examine the animal and its environment as holistically as possible.

2.2.1 Physical Assessment of Animal Welfare

Failure to cope with the environment can leave an animal susceptible to infection and disease (Broom, 1986), which often manifests in physical signs. One such sign is the animal's body condition (Broom & Fraser, 2007). The body condition score of an animal can inform the observer whether the animal is at an ideal weight, underweight, or overweight. Being underweight can be due simply to lack of appropriate diet, or to a more complicated issue, such as disease or a hormonal imbalance. Regardless of cause, a malnourished animal is expected to experience negative states such as hunger or malaise, thus indicating poor welfare (Fraser et al., 1997).

Other measures of physical health are illness and injury. A physical exam can provide insight into an animal's state of welfare by revealing obvious signs of illness, such as coughing or nasal discharge. Presence of injury may also be detected in this manner. These indicators of welfare may also shed light on the quality of the animal's environment. For example, higher instances of intestinal parasites have been associated with certain flooring types (Kochanowski et al., 2017) and access to the outdoors (Permin et al., 1999). Additionally, flooring type has been associated with instances of injury in dairy cattle (Mulling et al., 2006; Bicalho & Oikonomou, 2013). Examining an animal's environment, therefore, can contribute to the overall assessment of welfare.

Growth and reproduction are also used as welfare indicators. When an animal experiences frequent or prolonged stress, energy that would normally be allocated to processes such as growth or reproduction is needed to cope with that stress by maintaining homeostasis (Moberg, 2000). Difficulty coping can result in reduced growth rate (i.e., Hemsworth et al., 1981; Mitlohner et al., 2001), decreased production (i.e., Rushen et al., 1999; Waiblinger et al., 2002; Barnett et al., 1992), and reduced reproductive fitness (see Einarsson et al., 2008; Dobson et al., 2012; and Tilbrook et al., 2000 for reviews). While there are many physical means to assess welfare, many more clues to the welfare state of the animal lie beneath the surface.

2.2.2 Physiological Assessment of Animal Welfare

Animals maintain homeostasis using two systems: the nervous system (NS) and the endocrine system. The nervous system utilizes neurons to deliver electrical impulses to the brain via the spinal cord. One component of the nervous system, the sympathetic nervous system (SNS), prepares the body for stress-related activities in what is commonly referred to as the "fight or flight" response (Raven et al., 2008). Both positive and negative stimuli can activate the SNS (Yeates & Main, 2008), leading to the release of catecholamines such as epinephrine (adrenaline) and norepinephrine (noradrenaline) (Losos et al., 2008). These hormones have widespread and immediate effects, such as increased heart and respiration rates, and increased body temperature (Losos et al., 2008). The activation of the SNS can often be detected by measuring any of these effects. For example, Duncan and colleagues (1986) measured heart rate in broiler chickens in response to being caught either manually or by machine (Duncan et al., 1986). Heart rate, however, can be influenced by behavior, often confounding the cause of an observed change (Baldock et al., 1990). Measuring heart rate also cannot determine the type of stress an animal is experiencing (i.e., distress or eustress). Due to this limitation, heart rate variability (HRV) is often used instead of heart rate alone. An increase in HRV is associated with a relaxed affective state (e.g., Ali et al., 2017), while a decrease in HRV is associated with stress (e.g., Fenner et al., 2016; Gygax et al., 2008). In addition to cardiac factors, respiration rate can also be used to assess activation of the SNS. In one study, shaded feedlot cattle showed lower respiration rates than non-shaded cattle in response to heat stress (Mitlohner et al., 2001). Finally, body temperature can be used as an indicator of SNS activation. For example, it has been suggested that a rapid decrease in eye temperature occurs in response to pain due to sympathetically mediated vasoconstriction (Stewart et al., 2008). The aforementioned physiological metrics all require either handling the animal at the time of measurement, or the use of external equipment, which can be an unintended source of stress. Therefore, utilizing these metrics alone subjects researchers to the risk of inflated or confounded measures of stress. Additionally, SNS metrics serve to detect acute stress, thus they cannot be used to assess long-term welfare.

Alternatively, the endocrine system utilizes hormones released from various glands, delivered to target cells via the bloodstream, causing long-term and widespread responses (Losos et al., 2008). One class of hormones widely used in detection of stress is the glucocorticoids, which are released through activation of the hypothalamic-pituitary-adrenal axis (HPA-axis). Stress-inducing stimuli lead the hypothalamus to release corticotrophin-releasing hormone (CRH), activating the anterior lobe of the pituitary gland, which secretes adrenocorticotropic hormone (ACTH). ACTH then induces the adrenal cortex to release glucocorticoids (i.e., cortisol and corticosterone), which produce a wide-spread stress response (Hennessy, 2013; Lanoix & Plusquellec, 2013). Cortisol concentration is commonly collected via saliva, blood, urine, feces, or hair as a measure of stress. Salivary and plasma cortisol give an immediate picture of stress and increase in response to stressful stimuli (e.g., Young et al., 2012; Terlouw et al., 1991), however are more invasive to collect than some other methods. Urinary cortisol and fecal cortisol concentration represent roughly a 24-hour period of time. Collecting a spontaneous fecal sample, when possible, offers a less invasive method of cortisol measurement (Schatz & Palme, 2001). Urinary cortisol has been found to increase in response to stress yet is subject to variation associated with the circadian rhythm (Smith & French, 1997). Fecal cortisol concentration has been found to represent differing timeframes across different species (e.g., Harper & Austad, 2000; Whitten & Russell, 1998; and Schatz & Palme, 2001). Finally, hair cortisol concentration (HCC) represents a time period of weeks to months, which may offer the longest record of cortisol exposure (Sheriff et al., 2011); however, it can vary by hair color and body location (Heimburge et al., 2019). While glucocorticoid concentrations are useful measures of immediate, transient, and chronic stress, they, like SNS factors, cannot distinguish between eustress and distress.

Animals suffering from chronic stress have long been shown to have increased susceptibility to disease due to the negative effects of stress on the immune system (McEwen & Seeman, 1999). One promising way to measure such effects is the concentration of secretory immunoglobulin A (sIgA). IgA is present at mucosal surfaces, and thus plays an important role in the body's first line of defense against infection (Campos-Rodriguez et al., 2013; Staley et al., 2018). When the body experiences an infection, production of IgA is upregulated, which leads to an increase in sIgA in the feces. sIgA production can be mediated by HPA-axis activation. When glucocorticoids are released from the adrenal cortex, they induce proliferation of immune cells in the gut. This leads to an increase in IgA. However, chronic stress can cause a deficiency in IgA, either by the negative feedback loop in which glucocorticoids return to the glands of the HPA-axis and halt production of stress hormones, or by failure to return to normal hormone production. Salivary sIgA is used as a measure of short-term changes, and fecal sIgA can show changes in secretion over time. Therefore, a singular measure of sIgA can depict exposure to an acute stressor in a 24-hour period, and repeated measures can show how the animal is coping over time (Campos-Rodriguez et al., 2013; Staley et al., 2018). Several studies have found a negative correlation between stress and sIgA concentration in mice (e.g., Rammal et al., 2010), horses (e.g., Souza et al., 2010), pigs (Royo et al., 2005), and humans (Deinzer et al., 2000). However, others have found an upregulation in sIgA in response to acute stress (Jones et al., 2001; Reyna-Garfias et al., 2010). Due to the relative novelty of this metric and conflicting results, it is important to validate its reliability along with more established measures of stress.

2.2.3 Behavioral Assessment of Animal Welfare

Often the first place to start when using behavior as an assessment of welfare is observing presence or absence of abnormal behaviors. An abnormal behavior can be defined as one that is different in pattern, frequency, or context from what is shown by most members of that species (Broom & Fraser, 2007). Lack of, or excessive normal behaviors, along with abnormal behaviors can be indicative of poor welfare (Broom, 1986). One such example of an abnormal behavior is a stereotypy, or a repetitive sequence of movements with no obvious purpose (Broom & Fraser, 2007). Stereotypic behaviors often develop in order cope with stress, then persist and become stereotypies with the repetition of that stress over time (Pitman, 1989; Broom 1991). Stereotypies have been observed as potential measures of stress in many species, including horses (Waters et al., 2002; Young et al., 2012), zoo animals (Mallapur & Chellam, 2002; Greco et al., 2017), cattle (Schneider et al., 2020), swine (Terlouw et al., 1991a; Schouten et al., 1991; Tatemoto et al., 2020), and dogs (Mugenda et al., 2019; Tuozzi et al., 2021; Schipper et al., 2008). While stereotypies can certainly be indicative of negative affective states, instances of them are often variable, thus they must be utilized in concordance with other, more reliable measures of welfare (see review by Mason, 1991). Other abnormal behaviors can include self-mutilation (e.g., paw chewing in dogs or feather plucking in birds), coprophagy or ingesting of inappropriate objects, and hyperphagia or polydipsia (Broom & Fraser, 2007).

Another behavioral measure of welfare is the time budget of an animal. An animal's time budget is a catalogue of how an animal spends its time within a certain period. Alterations to an animal's normal time budget that suggest impaired welfare can include reduced or increased activity, reduced or increased resting, changes in levels of aggression with conspecifics, changes in environmental manipulation, or changes in instances of abnormal behavior (Pritchett et al., 2003; Schipper et al., 2008; Fureix & Meagher, 2015; Benhajali et al., 2008). Development of normal time budgets is imperative in order to create a standard ethogram of behavior for study species. Finally, response to stimuli is indicative of an animal's experience with said stimulus. Behaviors such as avoidance or aggression can suggest negative experiences, while affiliative behaviors suggest positive prior experience. Ethograms, or catalogues of defined behaviors, are often used as behavioral measures of stress in response to stimuli. There are many examples in the literature of animals avoiding or seeking certain stimuli after prior experience. Hens avoided completing a task which would result in food in order to avoid cage cleaning (Rutter & Duncan, 1992). In another study, gilts in an "unpleasant handling" treatment avoided human experimenters more than those in the "pleasant handling" treatment (Hemsworth et al., 1981). Cattle have shown a reluctance to enter a location in which aversive stimuli have occurred, such as use of an electric prod or hitting and shouting from a human handler (Pajor et al., 2003).

In summary, due to factors such as subject variation, predisposition to confound, and metric novelty, it is imperative to utilize multiple modes of stress measurement in order to accurately assess animal welfare.

2.3 Scientific Assessment of Welfare in Dogs

While much of animal welfare science has been studied in livestock and wild animals, a significant amount of research has been done on domestic dogs, particularly those in a kenneled environment. It is important to assess the welfare of kenneled dogs as most dogs will likely be placed in a kennel environment at some point in their life either temporarily, such as a in boarding kennel, or long-term, such as in a shelter. As dogs have been bred for centuries to form bonds with humans, there is an ethical obligation to ensure their needs are met, especially when they must be placed in an unnatural environment such as a kennel.

2.3.1 Physical Assessment of Welfare in Dogs

In dogs, many of the above-mentioned physical metrics can be used as indicators of welfare, such as body condition score (BCS) (Stella et al., 2018; Kovacs et al., 2005; Rooney et al., 2009), presence or absence of injury or disease or growth rate. Coat quality and tear staining have also been used as indicators of physical health (Stella et al., 2018; 2019; Mugenda et al., 2019; Barnard et al., 2016), although findings are variable. As many domestic dogs are sterilized, reproduction is not often a feasible means of welfare assessment, except in the case of dogs used for breeding. With the latter, some offspring health metrics can be indicative of the welfare of the dam. For example, prenatal stress has resulted in lower adrenal weights in blue fox cubs (Braastad et al., 1998; Osadchuk et al., 2001; 2004), which can suggest dysregulation of the HPA-axis. Additionally, in rats, dams subjected to stress during pregnancy have aborted or lost whole litters shortly after birth when control rats did not (Patin et al., 2002). The same study found lower birth weights in pups and altered maternal behaviors in stressed dams (Patin et al., 2002). Finally, while environmental factors are not direct measures of welfare, assessing the environment in addition to animal-based metrics can aid in gaining a holistic picture of animal welfare. If certain aspects of the environment are substandard, welfare may be negatively impacted. For example, some types of flooring have been shown to vary in pathogen load after cleaning (Stella et al., 2018). Additionally, flooring that remains wet has the potential to cause foot sores (Jennings, 1991 as cited by Rooney et al., 2009). While pen size requirements are present for facilities in which dogs are kenneled, some have found pen size to influence activity levels (Hubrecht et al., 1992; see Taylor & Mills, 2007 for review). If requirements set forth by governing bodies that oversee facilities in which dogs are kenneled are not developed according to scientific findings, this may have implications for dog welfare. Thus, while measures such as flooring type, kennel cleanliness, or pen size may not be used to measure welfare directly, they may be considered in addition to direct measures in order to indicate potential hinderances to welfare.

2.3.2 Physiological Assessment of Welfare in Dogs

Many physiological measures of welfare utilized in livestock are used in dogs. For example, heart rate in dogs has been found to increase in response to both stressful stimuli and arousal (e.g., Beerda et al., 1998; Zupan et al., 2016), and decrease in response to perceived positive stimuli (e.g., Handlin et al., 2011). Heart rate variability has also been widely used in dog studies. For example, Zupan and colleagues (2016) suggested positive emotional state to be associated with parasympathetic deactivation as measured by a decrease in high frequency range (Zupan et al., 2016). In addition to heart rate, another measure of the activation of the sympathetic nervous system is respiration rate. In one example, canine patients in a veterinary clinic were found to have a decrease in respiration rate in response to harp therapy (Boone & Quelch, 2003).

A more widely used physiological stress metric in dogs is cortisol concentration. As with livestock, cortisol concentration can be collected via saliva, blood plasma, urine, feces, and hair. Salivary cortisol has been shown to increase in response to stress-inducing stimuli in dogs (Morrow et al., 2015; Dreschel & Granger, 2005). However, saliva collection poses challenges in welfare assessment as some small dog breeds often do not produce sufficient saliva for analysis, and dogs, like humans, can experience "dry mouth" in response to stress. Saliva collection is also invasive, meaning it must be done quickly to prevent stress of collection from confounding the stimulus being measured. Similar results have been found in plasma cortisol (Tuber et al., 1996). Collection of plasma is also invasive, perhaps more so as it requires restraint, and can exceed the amount of time required to collect saliva. While both salivary and plasma cortisol present challenges with collection and assessment, they offer an immediate picture of stress induced HPA-axis activation.

Collection of urinary and fecal cortisol offer a less invasive approach and slightly longer-term measurement. Rooney and colleagues (2007) examined the differences in urinary cortisol between dogs habituated and not habituated to kenneling (Rooney et al., 2007). Non-habituated dogs had significantly higher cortisol to creatinine ratios than habituated dogs after ten days of kenneling (Rooney et al., 2007). Dogs in shelters have been found to have higher urinary cortisol levels than dogs in home environments (Stephen & Ledger, 2006). Additionally, fecal cortisol has been validated against urinary cortisol in dogs, with the peak concentration occurring 24 hours after administration of cortisol (Schatz & Palme, 2001). Both urinary and fecal cortisol offer a non-invasive, reliable measure of stress over roughly a 24-hour period. Challenges with these methods include confounding from possible sample contamination, diurnal patterns, or activity level (Polgar et al., 2019).

As for long-term, chronic stress measurement, researchers are increasingly turning to hair cortisol concentration (HCC) in dogs. HCC measurement has been validated against salivary, urinary, and fecal cortisol (Accorsi et al., 2008; Bryan et al., 2013), and found to be less variable over time than the former (Bryan et al., 2013). As cortisol accumulates in the hair over the period of growth, a depiction of stress over time can be acquired. Despite promising features, HCC is a relatively new stress metric and some conflicting results have been found. For example, lower HCC concentrations were found to be associated with psychosocial stressors, yet higher HCC concentrations were found to be associated with anxiety in dogs (Packer et al., 2019). Additionally, HCC has been shown to vary with hair color (Bennett & Hayssen, 2010), so caution must be taken when interpreting results. Finally, as mentioned above, metrics of HPA activation provide a great deal of information about physiological welfare but cannot differentiate between positive (eu-) and negative (di-) stress.

Studies of immune function in dogs provide measures and valence of stress. Lymphocyte proliferation has been found to increase in socially and spatially restricted dogs with experimentally induced bad weather (Beerda et al., 1999a). Fecal sIgA has been also validated in dogs (Tress et al., 2006), however there are conflicting results. One study found sIgA to increase in puppies after several stressful tasks yet decrease in adults of the same breed after 4 minutes of intense training (Svobodova et al., 2014). While acute stress can lead to immunoenhancement, chronic stress may cause immunosuppression (Hekman et al., 2014). sIgA was found to increase after separation from a pen mate in sub-adult and adult dogs housed in a shelter (Walker et al., 2014). One study found that breeding dams had significantly lower fecal sIgA concentrations during their second month of lactation compared to the first, yet fecal sIgA concentrations during pregnancy did not differ from those of control dogs (Grellet et al., 2014). Another study; however, found no significant difference in fecal sIgA across timepoints spanning 180 days when measuring stress in hunting dogs (Zannoni et al., 2020). Additionally, variations in sIgA across breeds have been found (Peters et al., 2004). Together, cortisol and sIgA can be used to measure stress and determine its valence.

2.3.3 Behavioral Assessment of Welfare in Dogs

Behavior of the domestic dog has been studied for quite some time, leaving researchers with a strong understanding of normal and abnormal dog behavior. Along with physiological metrics, behavioral metrics of welfare are integral to assessing welfare in dogs, especially those housed in kennels. However, behavior of dogs has been shown to be highly variable. Factors such as age, sex, size, breed, early life experiences, and temperament influence how dogs cope with their environment and stress inducing stimuli. Age has been shown to have a negative linear relationship with activity level (Wallis et al., 2020). Additionally, left pawedness has even been associated with higher expression of stress-related behaviors (Barnard et al., 2018). As dogs are highly individual, their behavior must be measured concurrently with physical and physiological metrics of welfare.

Similarly to livestock, time budgets of dogs can be examined as a welfare metric. Activity levels have been shown to vary in accordance with pen size (Taylor & Mills, 2007). Additionally, Schipper and colleagues found activity to increase after providing dogs with nutritional enrichment (Schipper et al., 2015). While the time budgets of dogs will vary across lifestyle, housing, breed, and individual, comparing baseline time budgets to those collected after an experimental change can provide valuable information regarding effects of the change on dog welfare.

Often, abnormal behavior, such as stereotypies, is used as an indicator of poor welfare in kenneled dogs. Shelter dogs that experienced regular walks showed a decrease in stereotypic behavior (Cafazzo et al., 2014). Additionally, higher instances of repetitive behavior have been shown in dogs housed in small, indoor kennels as opposed to dogs group-housed in environmentally enriched outdoor kennels (Beerda et al., 1999b) and circling increased in response to a slamming door (Beerda et al., 2000). Dogs regularly performing stereotypies were found to maintain the behavior after an owner-provided consequence (Hall et al., 2015). Finally, dogs exhibiting stereotypies without stimulation have been shown to respond atypically to stress

(Denham et al., 2014). While stereotypies can certainly indicate a stressful state in dogs, it is important to take their context into account. A difference between repetitive behavior in response to arousal and unprovoked stereotypical behavior has been suggested (Denham et al., 2014).

Other stress-related behaviors, such as calming and appeasement signals, and fear-based behaviors can be used to gauge dog welfare. When stressed, dogs will often display calming behaviors, such as lip-licking, paw lifting, yawning, body shaking (Beerda et al., 1997; 1998). To show submission, dogs will expose their bellies, lip-lick, paw lift, hold their ears back, or tuck their tails (Firnkes et al., 2017). At times, a fearful dog may show aggression, such as raised hackles, growling, or snapping (Bauer et al., 2017). Alternatively, a dog experiencing a positive affective state will display affiliative and relaxed behaviors, such as approaching and soliciting interactions, or adopting key postures such as a relaxed mouth, relaxed ears, and a wagging tail (Bauer et al., 2017). Understanding species-specific behaviors of dogs can assist in the determination of their affective states, thus providing information about their welfare.

Together, physical, physiological, and behavioral metrics of dog welfare can be utilized to assess the welfare of kenneled dogs. Greater knowledge about and validation of such measures allows for the evaluation of factors affecting welfare in dogs, particularly those housed in kennels.

2.4 Welfare Findings in Commercial Breeding Kennels

Historically, the welfare of dogs kept for commercial breeding has been understudied. However, some research is beginning to emerge. Before discussing these findings, it is important to note that the terminology for commercial dog breeding varies in published scientific literature and definitions are not always consistent. Terms such as “puppy farms”, “commercial breeding establishments”, and “puppy mills” are often used to describe large-scale and/or for-profit dog breeding. While there are no established definitions for the terms, “puppy mill” or “puppy farm,” they typically refer to establishments that operate under no regulation or oversight (Croney, 2019). Alternatively, in the U.S., a commercial breeding (CB) kennel is one that is required to operate according to requirements set forth by governing bodies at the local and national levels in order to maintain good standing. CB kennels are sometimes referred to as a commercial breeding establishment (CBE) in European studies.

One of the first studies relating to this population of dogs focused on the welfare of dogs believed to have formerly been used for commercial breeding and utilized the Canine Behavioral Assessment and Research Questionnaire (C-BARQ) in the form of an owner survey. Authors compared C-BARQ data on former breeding stock to a sample of pet dogs from the general population and found higher reported instances of health and behavior problems (e.g., fear/nervousness, compulsive behaviors, and house soiling) in the population of dogs formerly used for breeding (McMillan et al., 2011). A second study utilized similar methodology to compare puppies obtained from pet stores and those obtained from non-commercial breeders (McMillan et al., 2013). The authors found pet store puppies, particularly those which were intact, to have higher reports of owner-, stranger-, and dog-directed aggression than non-commercially bred puppies (McMillan et al., 2013). Other studies employing the C-BARQ have corroborated the findings of McMillan and colleagues, in that dogs originating in CBEs were reported to have higher instances of aggression, fear, and health issues compared to other pet dogs (Pirrone et al., 2016; Wauthier et al., 2018; Gray et al., 2016; see also review by McMillan; 2017).

A series of studies conducted onsite in CB kennels licensed by the United States Department of Agriculture (USDA) have found results that conflict with the above studies. In one study, kennel environments and dogs themselves were found to be clean, meaning there was little instance of visible kennel or body debris, and low instances of *E. coli* on pen floors after cleaning (Stella et al., 2018a). Dogs have been found to be physically healthy, with no significant health issues (Hurt, 2016; Bauer et al., 2017; Stella et al., 2019), have a similar incidence of periodontal disease to the pet dog population (Stella et al., 2018b), have few and minimal foot health issues (Stella et al., 2018a), and have ideal body condition (Hurt, 2016; Stella et al., 2018a; Stella et al., 2019). When assessing the behavior of dogs in CB kennels, however, results have been mixed. In a test evaluating the behavioral response to the approach of a human to the pen door, the majority of dogs showed affiliative behaviors (Mugenda et al., 2019; Stella et al., 2019; Bauer et al., 2017). In a study evaluating dogs in CB kennels that were candidates for rehoming, over 50% of dogs showed fearful responses during an extended approach test in which the pen door was opened, and an experimenter attempted to touch the dog (Stella et al., 2019).

There are several considerations as to why studies examining the welfare of dogs originating in CB kennels or used for commercial breeding yield conflicting results. Firstly, factors that can influence the manner in which owners respond to surveys include owner perception of unwanted dog behaviors (Pierrone et al., 2016) or bias regarding sourcing of the dog. Additionally, survey-based data does not allow for confirmation of dog origin, meaning dogs reported on may not have originated in commercial breeding facilities. While this observational data can serve as a basis for which future study may be directed, causation cannot be determined as measurements were not taken on dogs or in facilities themselves. Regarding the studies conducted in CB kennels, behavioral results have differed due to a difference in approach tests. While dogs show minimal fear in response to human approach outside of the pen (Mugenda et al., 2019; Stella et al., 2019; Bauer et al., 2017), the more invasive test conducted by Stella and colleagues (2019) elicited a higher proportion of fearful responses. The population of rehoming candidates studied there were likely older on average than the general population of dogs in CB kennels. It is possible that caretakers of dogs in the CB kennels tested were not aware of the need to socialize their puppies until more recently. Thus, older dogs in CB kennels may not have experienced socialization to new people during their sensitive periods. Scott (1962) stressed the importance of exposing puppies to novel stimuli in a positive manner during their critical period of socialization to avoid fearful responses as adults (Scott, 1962). Interestingly, another study found that dogs showing fearful responses to stranger approach were less fearful if a familiar caretaker was present (Bauer et al., 2017). This may suggest that the tested dogs had a positive relationship with their caretaker, since that person was able to act as a stress buffer for the fearful dogs. Further research is needed to assess the quality of caretaker interaction with dogs in CB kennels and its implication for welfare.

2.5 Effects of Human-Animal Interactions on Welfare

2.5.1 Effects of Human-Animal Interactions on Livestock and Animals Kept for Research

A number of studies have been conducted on human-animal interactions (HAIs) and many reviews have been published on the topic (e.g., Hemsworth, 2007; Rushen et al., 1999; Waiblinger et al, 2006; Zulkifli, 2013). Research has shown that livestock species are fearful of humans, and regular contact with humans can lead to a chronic stress response (Hemsworth, 2007). However, that fear may be reduced by positive interactions with stock people if they receive proper training (Hemsworth, 2007; Coleman & Hemsworth, 2014). Similarly, laboratory animals, depending on the species, may fear their human caretakers. Chronic activation of the HPA-axis due to fear can negatively affect welfare, (Poole, 1997; Prescott & Lidster, 2017). Therefore, the quality of HAIs can influence animal quality of life through creating positive or negative affective states and corresponding behavioral and physiological responses.

Behaviors indicative of fear of humans have been associated with negative health and production outcomes such as decreased egg laying (Barnett et al.,1992), increased mortality (Cransberg et al., 2000), and decreased feed to gain ratio (Hemsworth et al., 1994) in poultry. Similar results have been shown in swine. For example, gilts assigned to an unpleasant handling treatment had a lower growth rate, higher free corticosteroid concentration, and spent less time in proximity with an experimenter compared to those assigned a pleasant handling treatment (Hemsworth et al., 1981a). Many results of studies have supported these findings that aversive HAIs are associated with fear of humans and decreased growth and reproduction in swine (Gonyou et al., 1986; Hemsworth et al., 1986; Hemsworth et al., 1987). Again, studies in cattle have reported similar results. Hemsworth and colleagues (2000) found negative HAIs at a dairy farm to be negatively correlated with milk yield and approach to an experimenter, and positively correlated with milk cortisol (Hemsworth et al., 2000). While HAIs have been studied extensively in livestock, there are some similar findings in laboratory and zoo animals. For example, mice handled by the tail instead of less aversive methods were least likely to approach a handler and eliminated more during handling (Hurst & West, 2010). In a study involving several zoo animal species, greater amounts of nonverbal noise in attempts to call animals was negatively associated with animal approach, suggesting zookeeper noise may be aversive and is thus avoided (Carlstead, 2009). Additionally, lack of human interaction has been associated with fear of humans. For example, farmed silver foxes that were not handled at a young age showed more fearful behaviors in response to human approach than those that were handled (Pedersen & Jeppesen, 1990). While adverse or nonexistent HAIs are shown to negatively influence production levels and fear of humans many species, positive HAIs have been studied as well with results indicative of positive HAIs being beneficial to welfare.

Just as negative HAIs can influence animal welfare and production, positive HAIs have been shown to improve both aspects. One way in which positive HAIs are beneficial to welfare is through their influence on ease of handling. Veal calves that were stroked and allowed to suck their stockperson’s fingers during the fattening period approached both familiar and unfamiliar humans more than control calves (Lensink et al., 2000). The authors implied that the treatment could facilitate easier handling of calves (Lensink et al., 2000). Along with handling and production benefits, positive HAIs can aid in the mitigation of fear and stress in animals. For example, heifers experiencing early and prolonged handling consisting of brushing and leading with a halter were less reactive in flight-distance tests in the presence of a human (Boissy & Bouissou, 1988). Additionally, positively handled pregnant sows had lower daytime free plasma cortisol and a greater immune response to an antigen than negatively handled sows (Pedersen et al., 1998). In laboratory rats, the use of heterospecific play, or “rat tickling”, has been shown to decrease stress and increase affiliative behaviors (Cloutier et al., 2012; Burgdorf & Panksepp, 2001; LaFollette et al., 2017). In addition to laboratory and farm-housed animals, captive canids and felines have been studied as well. Silver fox vixens receiving HAIs accompanied by a high-quality food item spent more time in the front portion of their pens in response to human approach than those who did not receive the food item (Bakken, 1998). Finally, shelter cats that received a gentling treatment were more likely to display behaviors consistent with positive affective states, have higher sIgA values, and lower instances of upper respiratory diseases (Gourkow et al., 2014). Additionally, those experiencing a predictable schedule and minimal noise in the environment showed less sickness and hiding behaviors, more affiliative behaviors, and a shorter latency to interact with a stranger than those experiencing an unpredictable environment (Stella et al., 2014). These behavioral and physiological findings support the claim that positive HAIs can help to decrease fear of humans, thus decreasing the activation of the HPA-axis in response to human interaction. The findings from these studies serve as encouragement to not simply ensure the absence of poor welfare, but to strive for positive welfare for the animals used and cared for by society.

One important aspect contributing to the quality of HAIs is the attitude of the human. Attitude can be defined as a general evaluation on an object, assumed to be derived from inclinations, feelings, bias, preconceived notions, ideas, and convictions associated with that object (Thurstone, 1929). In the case of HAIs, a person’s attitude toward an animal in his or her care can be determined by the attributes of the animal, personal characteristics and beliefs, cultural factors, and experience (their own or that of another person) with animals (Serpell, 2004). Several studies in livestock have found evidence supporting correlations between stockperson attitude, stockperson behavior, and animal stress metrics. The general consensus in the literature describes a positive feedback loop between stockperson attitude and animal welfare. Those that hold negative attitudes toward the animals in their care tend to treat them more aversively than those with positive attitudes (Hemsworth et al., 1989). These negative interactions lead to the animals experiencing increased fear of humans (Hemsworth et al., 1986; 1987; 1994) which can make caring for them more challenging. Increased challenges in husbandry and handling can then lead to worsening attitudes toward the animals, which perpetuates the cycle. This process impairs animal welfare in that it induces chronic fear of humans, which leads to chronic activation of the HPA-axis (Gonyou et al., 1986; Hemsworth et al., 2000; Cransberg et al., 2000). One study done by Waiblinger and colleagues (2002) examined the relationship between personal characteristics of stockpersons, stockperson attitude, HAIs with dairy cattle, the behavior of cows during milking, and a human approach test (Waiblinger et al., 2002). Positive general attitudes towards cows were associated with both positive behaviors directed at cows and positive attitudes toward interacting with cows. Alternatively, negative general attitudes were significantly associated with aversive interactions, such as punishing a cow for kicking (Waiblinger et al., 2002), while positive HAIs were correlated with lower flight distance (Waiblinger et al., 2002). Hemsworth and colleagues (2000) reported similar results: positive stockperson attitude toward dairy cattle was found to be negatively associated with aversive tactile handling techniques, and those techniques were in turn negatively associated with human approach (Hemsworth et al., 2000). Another study on chickens found positive stockperson attitude toward chickens to be associated with lower levels of withdrawal during experimenter approach (Cransberg et al., 2000). Similar results have been found in zoo animals (Carlstead, 2009; Ward & Melfi, 2015).

Understanding the relationships between human attitude, HAIs, and measures of animal welfare can lead to interventions aimed at improving HAIs and in turn improving animal welfare. Positive attitudes toward the improvement of animal welfare have been associated with higher productivity (Kauppinen et al., 2012). Cognitive behavioral interventions designed to improve stockpersons' attitudes toward pigs led to increases in positive attitudes, reduction of aversive handling, reduction of pig fear towards humans, and increases in productivity (Hemsworth et al., 1994; Coleman et al., 2000). These behavioral interventions were aimed at modifying stockperson beliefs regarding animal sensitivity and the effects of aversive handling techniques on ease of handling and productivity (Coleman et al., 2000). The research shows that human attitude influences HAIs, and those HAIs in turn influence animal welfare, especially in livestock. Currently, training programs, such as "ProHand", are utilized in the livestock industry to adjust stockperson attitudes in order to improve human-livestock interactions, thus reducing fear and improving welfare (Hemsworth et al., 2002). If HAIs are important to the welfare of livestock, it is logical to hypothesize that they might affect the welfare of species with longer domestication histories, such as the dog.

2.5.2 Effects of Human-Animal Interactions on the Welfare of Dogs

Canids and humans have coevolved for as many as 135,000 years (Vila et al., 1997; Schleidt & Shalter, 2003), with the first evidence of the divergence of dogs from wolves occurring up to ~30,000 years ago (Druzhkova et al., 2013; Germonpre et al., 2015; Thalman et al., 2013), and domestication beginning around 14,000-15,000 years ago (Benecke, 1987; Savoleinen et al., 2002). Thus, today's humans and dogs have a long, rich history of interaction and cooperation. In fact, dogs have been shown to be more adept at communication with humans than primates (Hare & Tomasello, 2005; Kirchhofer et al., 2012; Gomez, 2005) and wolves (Hare et al., 2002; Miklosi et al., 2003), pointing to a possible selection for communication with humans over their evolutionary history (Hare & Tomasello, 2005). Therefore, the quality of HAIs between dogs and people is likely to be important to their welfare.

HAIs have not been studied quite as extensively in dogs as they have in livestock. However, there are several studies to consider. One study examining oxytocin release in response to mutual gazing behavior found a positive feedback loop in dog-human dyads, which the authors suggested could result in social reward, in turn facilitating inter-species bonding (Nagasawa et al., 2015). Beyond gazing, dogs have been shown to display behaviors indicative of attachment bonds with their human owners based on a modified version of Ainsworth's Strange Situation Test (Topal et al., 1998; 2005). Briefly, in the Strange Situation Test (SST), a caregiver and infant enter a novel room, followed by stranger. The caregiver then leaves for a short period of time and returns. Next, the stranger and caregiver both leave in succession, leaving the infant alone in the room. Finally, the stranger returns, followed by the caregiver. The infant's behavior is observed, with particular attention paid to reunions between the infant and caregiver (Van Rosmalen et al., 2015). The SST was adapted by Topal and colleagues (1998) for use with dogs. Authors found dogs to play and explore more in the presence of the owners, spend more time in proximity to the door when the owners were absent, and demonstrate higher levels of contact-seeking upon owner return compared with stranger return, suggesting that attachment behavior was elicited by the SST (Topal et al., 1998). Additionally, in a novel pen, dogs spent more time with their caretakers than their kennelmates, and presence of a caretaker appeared to mitigate the glucocorticoid increase in response to being placed in a novel environment (Tuber et al., 1996). The presence of owners or familiar caretakers has also been shown to reduce stress responses in dogs in several studies (e.g., Gacsi et al., 2013; Mariti et al., 2013; Pettijohn et al., 1977).

Several interventions in the form of systematic, high-quality HAIs intended to improve dog welfare have been studied in kenneled dogs, many of them in animal shelters. In one study, plasma cortisol decreased after a 30-minute positive interaction with a human in shelter dogs (Shiverdecker et al., 2013). This result was further supported by another study that found salivary cortisol to decrease on the third day of shelter housing in dogs that received a 45-min positive human interaction period (Coppola et al., 2006). Additionally, repeated 20-minute positive interactions combined with a high-quality diet decreased anxious behavior in response to a stranger (Hennessy et al., 2002). Further supporting these results, positive human interaction and training via positive reinforcement sessions were associated with improvements in sociability in shelter dogs (Bergamasco et al., 2010). Even just 2-minutes of a daily positive interaction led to increased time in the front portion of the pen in response to approach by both familiar and unfamiliar people in shelter dogs (Conley et al., 2014).

While these results suggest that incorporating positive HAIs into husbandry routines can improve welfare in kenneled dogs, conflicting results have also been found. In the above-mentioned study by Hennessy and colleagues (2002), the positive human interaction program alone did not yield significant results (Hennessy et al., 2002). Additionally, a fifteen-minute petting session between a volunteer and shelter dogs did not result in a difference in salivary cortisol concentration (McGowan et al., 2018). In another study, experimenters either exercised or calmly interacted with shelter dogs, which resulted in an increase in back-and-forth locomotion after the interaction (Protopopova et al., 2018).

A possible cause for these conflicting results is the degree of familiarity between the dogs studied and the people performing the HAI interventions. Differences in how dogs respond to unfamiliar and familiar people have been found (Rehn et al., 2013; Gacsi et al., 2001; Kuhne et al., 2012), especially during stress-inducing events (Kerepesi et al., 2015), although the majority of these findings are in dogs in homes. It is possible that the above-mentioned interventions did not serve to improve welfare because HAIs intended to be positive may not have been positive for the dog. A forced interaction, although positive in nature, may have the unintended consequence of increasing stress in a dog that is fearful or in a state of high arousal. Additionally, the people performing them may have been unfamiliar to the study dogs or did not have strong bonds with them. In one study, shelter dogs were found to display more fear and appeasement behaviors in the presence of an unfamiliar experimenter than dogs living in homes, which the authors attributed to the shelter dogs' lack of bond with their caretakers (Barrera et al., 2010). Due to the fact that dogs in shelters do not share bonds with unfamiliar people, such as experimenters or caretakers with whom the dog does not have an established relationship, differences in behavior in response to familiar versus unfamiliar people may not mimic those of owned companion dogs. Because of the strong bonds dogs are capable of forming with humans, understanding the effects of human-animal interactions on dog welfare in kennels is imperative.

It is clear that positive HAIs have the potential to improve welfare in shelter dogs, although some study results are conflicting. While it is possible that these results can translate to dogs in CB kennels, it cannot be assumed. There are many ways in which shelter dogs differ from those in CB kennels. For example, the histories of dogs in shelters are often unknown, their length of stay in the shelter is often short relative to the time dogs are kept in CB kennels, and the dogs in shelters are often studied when the environment is novel to them. Another challenge to extrapolating results from shelter studies is that dogs are often adopted before any long-term effects can be measured. Additionally, the degree of familiarity of the caretaker may influence effects of HAIs and any interventions that are introduced. In most cases for dogs in CB kennels, their caretaker is likely more familiar than that of a shelter employee or volunteer. Because of these differences, the effects of HAIs and interventions on the welfare of dogs in CB kennels warrants investigation.

The current study therefore aimed to determine the effects of a brief, easy to implement positive caretaker interaction on physical, physiological, and behavioral indicators of dog welfare in CB kennels.

CHAPTER 3. EFFECTS OF POSITIVE CARETAKER INTERACTIONS ON DOG WELFARE IN COMMERCIAL BREEDING KENNELS

3.1 Ethics Statement

All procedures were reviewed and approved by the Purdue University Institutional Animal Care and Use Committee (PACUC #1802001693A003). Access to commercial breeding facilities, and permission to record video and collect data was granted by facility owners prior to the study.

3.2 Materials and Methods

3.2.1 Subjects and Facilities

The study was carried out in two Amish-owned commercial breeding kennels in the Midwestern U.S.: Facility 1 and Facility 2. Facility 1 was located in Indiana and Facility 2 was located in Michigan. Facility owners were recruited from a list of research volunteers and were selected based on their satisfaction of the following criteria: possession of at least 30 adult, female dogs; no prior, regular, positive caretaker interaction protocol, such as giving of treats; owner willingness to have security cameras present in the kennel for four weeks; owner commitment to completing a daily, positive caretaker interaction protocol for two weeks; and legal operation of the commercial breeding kennel as determined by the United States Department of Agriculture (USDA) and state of operation.

Focal dogs were selected for study upon satisfaction of the following criteria: pair- or group-housed bitches, over one year of age, not heavily pregnant (i.e., no less than two weeks pre-parturition), generally healthy, and small or medium breed. A total of 47 dogs between Facilities 1 and 2 met study criteria. The mean age of focal dogs was 3.02 $\pm$ 1.35 years (Facility 1: n=25, μ_{age} = 2.65 $\pm$ 1.08 years; Facility 2: n=22, μ_{age} = 3.45 $\pm$ 1.52 years) and 11 breeds were represented (see Table 3.1). Facility 1 housed 16 pens, with 10 focal pens. There were 2-4 dogs per pen, with 2-3 focal dogs per pen. Seven pens housed only female dogs, and three pens housed one male along with the female dogs. An immediate family member of the main caretaker performed the interaction in the afternoon, with a 2-hour range in start times. Facility 2 housed 43 pens, with 11 focal pens. Each focal pen contained two focal dogs and no non-focal dogs. The main caretaker performed the interaction in the morning, with a 30-minute range in start times.

Table 3.1. Description of focal dog breeds

Breed	**Sample Size**			**Percent of Total**
	Facility 1	**Facility 2**	**Total**	
Bichon Frise	n=5	n=0	n=5	10.6%
Bichon Frise/Shih Tzu Cross	n=2	n=0	n=2	4.26%
Cavalier King Charles Spaniel	n=0	n=3	n=3	6.38%
Cocker Spaniel	n=0	n=1	n=1	2.13%
Daschund	n=0	n=3	n=3	6.38%
Maltese	n=0	n=2	n=2	4.26%
Miniature American Shepherd	n=0	n=1	n=1	2.13%
Pomeranian	n=10	n=0	n=10	21.3%
Pug	n=0	n=3	n=3	6.38%
Shih Tzu	n=8	n=3	n=11	23.4%
Toy Poodle	n=0	n=6	n=6	12.8%
Total	n=25	n=22	n=47	100%

Experimental Groups

Each focal dog served as its own control. Twenty-one focal pens were pseudo-randomly assigned into two groups. One group (11 pens, n= 24 dogs) received a daily, two-minute, positive caretaker interaction with treats (CI). The other group (10 pens, n=23 dogs) received a daily treat from the caretaker without a positive interaction (TO). The same familiar caretaker completed both interactions daily at a consistent time for two weeks, apart from Sundays, when breeders did not work for religious reasons. All focal pens received either the two-minute, positive caretaker interaction (CI), or the treat-only interaction (TO). To avoid frustration of non-focal dogs in adjacent pens as a result of not receiving treats while others did, all non-focal dogs also received the TO interaction. Additionally, in Facility 1, focal pens facing each other received the same treatment, again to avoid effects of frustration. Half of the facility (i.e., front or back) was randomly assigned to the CI or TO group. In Facility 2, with the exception of one pen, all focal pens were in the same row (i.e., no focal pens faced each other). Both facilities had two rows of pens with an aisle in the middle (see Figure 3.1). The caretaker began the interactions with the first pen on one side and continued down that row in pen order, meaning pen 1 received the interaction, followed by pen 2, followed by pen 3, and so on, regardless of treatment. The caretaker was instructed to alternate the side of the facility and end of the row he or she began with from day to day (i.e., Day 1: begin with row 1, pen 1, Day 2: begin with row 2, pen 20, Day 3: begin with row 1, pen 10, etc. See Figure 3.1.).

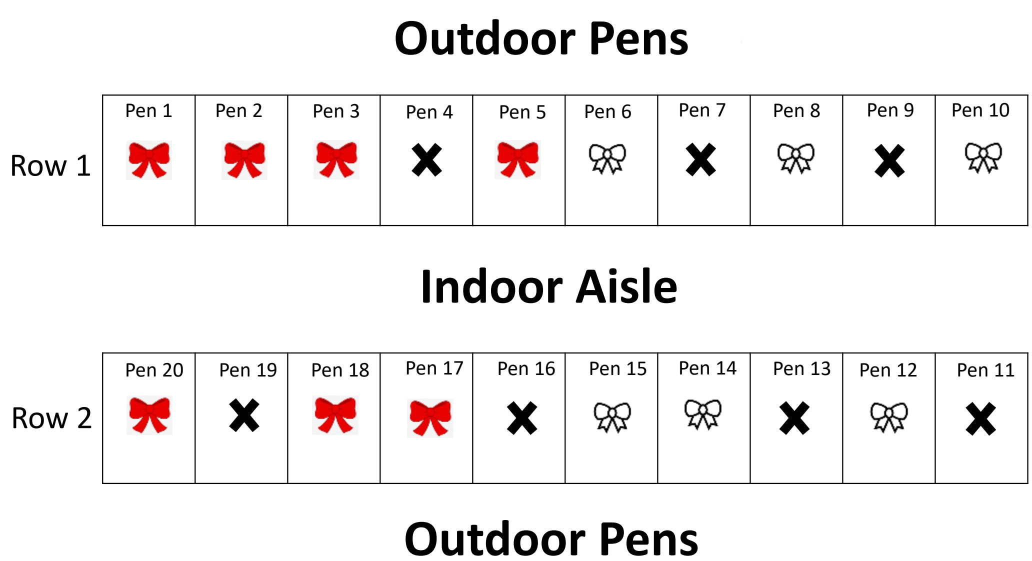


Figure 3.1. Experimental set-up example: Solid, red ribbons represent 2-minute caretaker interaction (CI) pens. Blank, white ribbons represent treat-only (TO) pens. Black X's represent non-focal pens. Caretakers were instructed to alternate the row (i.e., 1 or 2) and end of row (i.e., pens 1, 10, 11, or 20) they started with each day.

2-Minute Positive Caretaker Interaction (CI) with Treats

During the two-minute, positive CI, the familiar caretaker approached the pen, opened the door, and offered one treat (Bil Jac: Original Recipe Dog Treats) to each dog from his or her hand. If a dog did not accept a treat directly from the caretaker's hand, the caretaker placed the treat on the floor. After offering treats, the caretaker then set a stopwatch (VWR International, LLC., Pennsylvania, USA) for two minutes. During the two minutes, the caretaker was allowed to interact with the dogs the way he or she normally would (i.e., talking to or petting dogs) so long as it was in a quiet and gentle manner. The caretaker was allowed to sit or stand in front of the pen, depending on whether the pen was elevated or at ground level. In both facilities, dogs were free to exit the indoor portion of the pen to go to the outdoor portion of the pen for the entirety of the interaction. Regardless of whether dogs in the pen chose to remain in the indoor portion, the caretaker remained at the front of the pen with the door open for the full two minutes. Upon completion of the two minutes, the caretaker closed the pen door and moved on to the next pen. This was repeated for every pen in the CI group.

Treat-Only Interaction (TO)

During the TO interaction, the familiar caretaker approached the pen and, with the door closed, offered one treat (Bil Jac: Original Recipe Dog Treats) to each dog. The design of the pen doors at both facilities allowed the caretakers to hand treats to dogs through the gaps in the door. If dogs were at the front of the pen when the caretaker approached, he or she was allowed to directly hand a treat to each dog. If dogs were not at the front of the pen or did not take treats directly from the caretaker's hand, the caretaker dropped one treat per dog onto the pen floor. In one facility, the flooring type allowed for treats to fall through the floor to the area beneath the pen. In this case, the caretaker was instructed to place another treat on the pen floor if a treat fell. Once the caretaker had offered one treat per dog in a pen, he or she moved on to the next pen. This was repeated for every pen in the TO group.

3.2.2 Experimental Design

Protocol Overview

On Day -1 (setup), treatment pens were assigned, and focal dogs were selected and identified via marking with a non-toxic marking pen (Blue Squid, Hampshire, UK). Security cameras connected to a recording system (Swann NVR-87400, Victoria, AU) were set up in the indoor and outdoor portions of the pens for video recording of dog behavior. The following day (Day 0), baseline behavioral, physical, and physiological metrics of welfare were collected. Behavioral responses to approach of both an unfamiliar experimenter and the familiar caretaker were recorded, followed by collection of a naturally voided fecal sample for analysis of fecal secretory immunoglobulin a (sIgA), a hair sample for analysis of hair cortisol concentration (HCC), and physical health metrics (e.g., body condition score). On Days 1-14 (apart from Sundays), the caretaker performed the treatment. On Day 14 (post-treatment), after completion of the treatment, the same metrics were collected as on Day 0, with the exception of a hair sample as hair regrowth was insufficient for analysis by Day 14. Finally, on Day 28 (long-term), the same metrics as on Days 0 were collected to determine whether changes in metrics of welfare persisted after the interaction ended (see Figure 3.2).

W	Th	F	Sa	Su	M	T	W	Th	F	Sa	Su	M	T	W	Th		W	Th
-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	…	27	28
C	B	V	V					R	V	V			R	V	PV		R	L

Figure 3.2. Timeline of data collection. Camera setup (C) occurred on Day -1. Baseline metrics (B) were collected on Day 0. Remarking (R) occurred on Days 7, 12, and 27. Post treatment metrics (P) were collected on Day 14. Long-term metrics (L) were collected on Day 28. Video recording of the caretaker interaction and surrounding 4 hours (V) occurred on Days 1, 2, 8, 9, 13, and 14. The caretaker interaction occurred on Days 1-2, 4-9, and 11-14.

3.2.3 Data Collection

Focal Dog Selection and Identification

The study spanned twenty-nine days. On the first day (Day -1), facility owners received an overview of the study and signed consent forms. In both facilities, all dogs that met study criteria were chosen to be focal dogs. Focal pens were pseudo-randomly assigned to receive either the two-minute positive interaction or the treat-only interaction and identified with differently colored ribbons. Focal dogs that were not easily distinguishable from pen-mates received two uniquely shaped and colored lines down their back. Focal dogs that were easily distinguishable from pen mates were sham marked. With the cap on the marker, the marker was run down the back of the dog twice to mimic marking. Some studies have shown marking to impact behavior in social groups (Dennis et al., 2008). However sham marking ensured all dogs received the same handling while allowing for a speedier process and potentially reduced stress on the animals. Marking of focal dogs was repeated three more times throughout the study (Days 7, 12, and 27) to ensure dogs were always identifiable in video recordings and during approach tests.

Physical and Physiological Metrics

Upon arrival at the facility on Day 0, researchers collected focal dog feces for analysis of fecal secretory immunoglobulin A. In Facility 1, non-focal dogs in pens with focal dogs were fed a fecal marker of edible, non-toxic glitter mixed with baby food (Sulyn, Florida, USA) for differentiation of feces. This was repeated roughly twenty-four hours prior to sample collection at each timepoint. Due to the small number of non-focal dogs in pens, it was more time-efficient to mark the feces of non-focal dogs. This also allowed for consistent treatment of focal dogs between facilities as in Facility 2, each focal pen only housed focal dogs, so no dogs were fed a fecal marker. Feces were analyzed at the pen level. One fecal sample was collected for each focal dog (i.e., if the pen housed two focal dogs, two separate fecal samples were collected). In Facility 1, feces with fecal markers were avoided in order to ensure only focal dog feces were collected. Fecal samples were stored in a Styrofoam cooler with two ice packs until they were placed in a freezer at -20^{o} Celsius. Fecal samples were sent to Omaha’s Henry Doorly Zoo and Aquarium (Omaha, NE) for analysis. sIgA was extracted via sandwich ELISA in which IgA is bound to anti-Dog IgA antibody pre-adsorbed on microtiter wells. Unbound proteins and molecules were then washed off and biotinylated detection antibodies were added to the bound IgA. Strepavidin-conjugated horseradish peroxidase (SA-HRP) was used to catalyze the colorimetric reaction with a chromogenic substrate TMB (3,3', 5,5'-tetramethylbenzindine). The blue product produced by the reaction turned yellow after the addition of dilute sulfuric acid. The amount of IgA present in the sample was proportional to the absorbance of yellow product at 450nm, allowing for generation of a four-parameter standard curve. IgA concentration in samples could then be extrapolated from the curve and calculated from factoring sample dilutions (Hau et al., 2001; Bethyl Laboratories, Inc, Texas, USA).

After conclusion of the three approach tests, dogs were removed from pens individually for a physical exam, blood draw, and hair collection. The physical exam utilized an adapted version of Bauer and colleagues' (2017) physical portion of the FIDO (Figure 3.3). One experimenter (JR) completed all scoring of physical metrics.

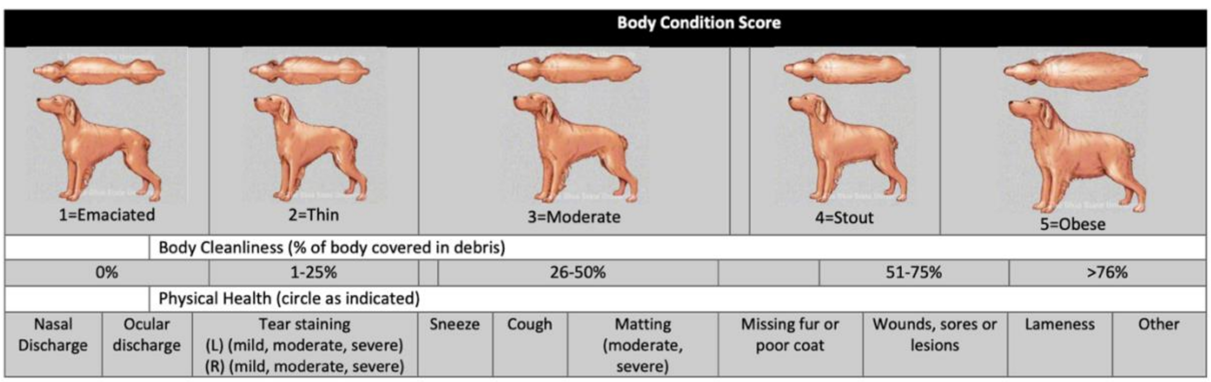


Figure 3. 3. Metrics of the Field Instantaneous Dog Observation Tool collected during the physical exam. Adapted from Bauer et al., 2017.

Dogs were restrained for collection of hair and blood using low-stress handling techniques. One mL of blood was collected from each dog for use in a separate study (unpublished). The experimenter drawing blood did not conduct any behavioral testing for the remainder of the study. Finally, about 50mg of hair was collected from above the base of the tail (by shaving an approximately 2in x 2in area) and stored in a small labeled manilla envelope for analysis of hair cortisol concentration (HCC). Hair samples were stored in a drawer at room temperature until shipped to The Endocrine Technologies Core (ETSC) at the Oregon National Primate Research Center (ONPRC). Hair cortisol levels were determined by EIA (Salimetrics, California, USA) using a modification of Davenport and colleagues' (2006) protocol. Hair samples were washed with isopropanol, drained through P8 filter paper (Thermo Fisher Scientific, Massachusetts, USA), and dried. Approximately 50mg of each hair sample was subsequently weighed into a reinforced microtube with five 4mm steel beads added for grinding. Samples were then ground (2 x 5min) using a Spex SamplePrep 5100 grinder (Spex, New Jersey, USA). One mL methanol was added to the ground hair samples and cortisol was extracted overnight with gentle shaking. Samples were then centrifuged to collect hair and supernatants were transferred to fresh tubes and evaporated until dry. Samples were reconstituted to 0.3mL of PBS and cortisol levels were determined by EIA. Recovery was determined at the same time as sample analysis and used to adjust final sample cortisol values. The assay range was 0.12ng/mL to 30.0 ng/mL and intra-assay variation was 3.0%. No inter-assay variation was determined for this analysis because all cortisol concentrations were determined in a single assay. Inter-assay variation for hair cortisol analysis in the ETC is <15% (ETSC; ONPRC).

Once collection of physical and physiological metrics was complete, the dog was returned to its pen. Physical exams and blood collection were repeated on Days 14 and 28. Hair collection from the same area (shave-reshave technique) was only repeated on Day 28 as not enough hair for collection had re-grown by Day 14.

Behavioral Metrics

Caretaker Interaction

The caretaker interaction (both CI and TO) was video recorded and scored continuously using the ethograms in Tables 3.2, 3.3 and 3.4 on days 1, 2, 8, 9, 13, and 14 to analyze how dogs responded to interacting with their caretaker. Additionally, video recording occurred on days 1, 2, 8, 9, 13, and 14 for four consecutive hours in order to detect changes in behavior surrounding the daily interaction using the ethogram in Table 3.2. Recording began one hour before the interaction took place and ended two hours after completion of the interaction (e.g. if the interaction occurred in the 9 o'clock hour every morning, recording began at 8:00 AM and ended at 12:00 PM). Instantaneous scoring of individual focal dogs took place every five minutes, for a total of 48 scans per day.

Human Approach Tests

On Day 0 (baseline metrics), an approach test (RYG) was conducted at the front of the indoor portion of each focal pen while dogs were free to move to the outdoor portions of their pens. In the approach test, adapted from the behavioral portion of Bauer et al.'s (2017) FIDO (Field Instantaneous Dog Observation Tool), an unfamiliar experimenter approached the front of the pen, maintaining a sideways facing orientation to the dog in order to avoid eye contact. The immediate response to approach of each dog in the pen was scored as red, yellow, or green (RYG) (Bauer et al., 2017). A red score was assigned to dogs that showed behaviors consistent with fight, flight, being frozen in place, or showing stereotypic behaviors; a green score encompassed affiliative or undisturbed behavior; and a yellow score included behaviors that were not clearly red or green, or ambivalent behaviors (i.e., approach and avoid) (Bauer et al., 2017). If a dog was not in the indoor portion of the pen, the response was recorded as "out". Scores were recorded live into a spreadsheet (Microsoft Excel) by a second experimenter with an iPad (Apple Inc.) and testing was video recorded on the security cameras for validation of live scores. At each time point, one experimenter approached and scored all focal pens. However, to keep the testers unfamiliar to the subjects, different experimenters were alternated. All experimenters were female. To comply with COVID-19 precautions, face masks were worn while in the facility but were removed during behavioral testing to avoid unintentionally eliciting fear or other emotional states that might have altered the dogs' behaviors.

To further determine effects of the caretaker interaction on response to an unfamiliar person, an extended approach test was conducted. Previous studies have found behavioral response to human approach to differ depending on intensity of the interaction (Stella et al., 2019; Barnard et al., 2021). Following the first RYG approach test, all focal dogs were confined to the indoor portions of their pens and given five minutes to acclimate. Depending on the facility's system for confining dogs indoors, non-focal dogs were either confined indoors with focal dogs (Facility 1) or retained the ability to enter and exit the indoor portion of their pens (Facility 2). Upon completion of the acclimation period, the extended approach test was conducted with each focal pen. The approach test (RYG+) was adapted for use with group-housed dogs from the behavioral portion of Stella and colleagues' Field Instantaneous Dog Observation Tool+ (FIDO+) (Stella et al., 2019). In this adapted approach test, the unfamiliar experimenter approached the front of the indoor portion of the pen in the same way as the initial RYG approach test, opened the pen door, extended her arm inside of the pen, and remained with her arm extended for one minute to allow assessment of latency to approach and behavioral response to the extended presence of an experimenter. The same RYG score as described above was given for the dogs' immediate reaction to three test stages: approach to the pen, opening of the door, and primary extension of the arm. If a dog initiated an interaction, the experimenter was allowed to gently scratch the dog on the chest. This extended test was scored live, and video recorded in the same manner as the initial approach test. The one-minute arm extension was later scored from video using an ethogram (Tables 3.3 and 3.4). The focal pens were divided into two groups and randomly assigned one of two unfamiliar experimenters to avoid observer effect. To maintain unfamiliarity, each experimenter only tested each group of pens once (see Figure 3.4).

To determine whether dogs' behavioral responses to their familiar caretaker who performed the daily interaction had changed, the 3-step approach test was repeated with the caretaker. This was done after the unfamiliar experimenter approach to avoid any potential stress buffering effect the caretaker might have on behavioral response to an unfamiliar experimenter. Following the RYG+ testing with an unfamiliar experimenter, dogs were given a minimum of five minutes to settle. After the settle period, the familiar caretaker performed the actions of the RYG+ but did not assign scores to the dogs. In order to avoid any effects an unfamiliar person would have on dogs' behavioral responses to their caretakers, researchers remained out of sight during the caretaker RYG+. Testing was video recorded via security cameras and scored later from video. All three approach tests were repeated on Day 14 (post-treatment metrics) and Day 28 (long-term metrics) (Figure 3.4).

RYG Testing Schedule

Day 0	Day 14	Day 28
Experimenter 1	Experimenter 2	Experimenter 3

sRYG+ Testing Schedule

Testing Group	Day 0	Day 14	Day 28
1	Experimenter 1	Experimenter 2	Experimenter 3
2	Experimenter 2	Experimenter 3	Experimenter 4

Figure 3.4. Experimenter schedule for RYG and RYG+ testing. As experimenter 1 performed blood collection on Day 0, she did not complete any behavior testing on the following days.

Table 3.2. Ethogram of behaviors coded for analysis of behaviors of focal dogs surrounding the daily interaction. Behaviors notated with letters (abcdef) were mutually exclusive to each other. Behaviors were scored instantaneously every 5 minutes over 4 hours for a total of 48 scans per focal dog per day.

AREA	---
[a]Outside	Dog is in the outside run
[a]Inside	Dog is in the inside run
[a]Between	Dog is between the outside and inside runs
Confined to indoor pen	Dog is in the inside run without access to the outside
Confined to outdoor pen	Dog is in the outside run without access to the inside
[a]Missing	Dog is not in the run
LOCATION (Indoor and Outdoor)	---
[b]Back	Dog has at least 50% of body in the back half of the kennel (furthest from gate)
[b]Front	Dog has at least 50% of body in the front half of the kennel (closest to gate)
[b]Middle	Dog in the middle of the kennel and not clearly on either side of the center line
LOCATION OF KENNELMATE	---
[c]Near	Within 1 dog length of at least 1 kennelmate (Flint et al., 2022)
[c]Far	Further than 1 dog length from kennelmates (Flint et al., 2022)
POSTURE	---
[d]Lie	Chest and rear end in contact with the ground (Flint et al., 2022)
[d]Sit	Front legs straight, rear end lowered, and resting on haunches or ground (Flint et al., 2022)

Table 3.2 Continued

[d]Play bow	Front legs lie down while hind legs remain standing so that anterior is lowered while posterior is raised. Oriented toward caretaker or kennelmate (Protopopova et al., 2014; Bauer et al., 2017)
[d]Stand	Upright on all four legs (Flint et al., 2022)
[d]Rear	Stand on hind legs with front paws off the ground (Flint et al., 2022)
BEHAVIORS	---
[e]Slow Locomotion	Walk, 4 beat gait and 3 feet on the ground at one time (Demirbas et al., 2017)
[e]Rapid locomotion	Trot, 2 beat gait, diagonally opposite legs move together (Demirbas et al., 2017)
[e]Idle	Standing, sitting, rearing, or lying with head up, and not performing any other listed behavior (Flint et al., 2022)
Vigilant	Standing, sitting, rearing, or lying with head up, and gazed fixed towards the outside of the pen (Flint et al., 2022)
Rest	Lying down with the head on the ground without any obvious orientation toward the physical or social environment (Flint et al., 2022)
Explore	Sniffing, scratching, digging, chewing, biting, or licking at parts of the physical environment including ground, walls or gates (excluding enrichment) (Flint et al., 2022)
Interact with Enrichment	Any action directed towards environmental enrichment such as toys or bones, including sniffing, chewing, biting, shaking from side to

Table 3.2 Continued

	side, tugging, scratching or batting with the paw, chasing, rolling balls, and tossing using the mouth (Flint et al., 2022)
Affiliative social interaction	Dog interacts in a manner intended to facilitate group cohesion and bonding with one or more kennelmate(s) (i.e. sniff, lick, play).
Agonistic social interaction	Dog interacts in a submissive or threatening manner with one or more kennelmate(s) (i.e. belly-up, low posture; or growl, lunge, bite threat), hackles may be raised
Bark	Short, loud, low frequency vocalization (Protopopova et al., 2014; McGreevy et al., 2012; Ng et al., 2014; Hewison et al., 2014; Beerda et al., 1998; Bauer et al., 2017)
Growl	Throaty, low, rumbling vocalization (Protopopova et al., 2014; McGreevy et al., 2012; Ng et al., 2014; Hewison et al., 2014; Beerda et al., 1998; Bauer et al., 2017)
Whine	Cycling high vocalization (Protopopova et al., 2014; McGreevy et al., 2012; Ng et al., 2014; Beerda et al., 1998)
Howl	Prolonged high-amplitude vocalization of varying pitch, lips drawn together while exhaling (Protopopova et al., 2014)
Eat	Head lowered into food bowl while consuming food (Flint et al., 2022)
Drink	Licking at water source while ingesting water (Flint et al., 2022)

Table 3.2 Continued

Groom	Action of cleaning the body surface by licking, nibbling, picking, rubbing, and scratching directed towards the dog's own body (Flint et al., 2022)
Repetitive	Dog repeatedly travels on a fixed route (>3 times) (Flint et al., 2022)
Jump on/paw at door	Paw(s) make(s) contact with pen door (Protopopova et al., 2014)
Coprophagy	Biting, licking or chewing of own/other dog's feces
Urinate	Squat, leg raise or lean with elimination of urine (Flint et al., 2022)
Defecate	Hind end lowered, back arched, and tail held out with elimination of feces (Flint et al., 2022)
Wall climbing	Dog attempts to scale wall or pen door
Enter	Dog moves from outdoor to indoor portion of pen
Exit	Dog moves from indoor to outdoor portion of pen
Oral behaviors	Tongue out, lip lick, snout licking, swallowing, or yawn (Hewison et al., 2014; Ng et al., 2014; Beerda et al., 1998)
Destructive oral	Chewing or biting equipment and environment (Demirbas et al., 2017)
Stretch	Extending body while one or more front/hind legs remain stationary (Protopopova et al., 2014; Ng et al., 2014; Beerda et al., 1998)
Shake off	Rapid, repeated motion of head and/or body back and forth (Protopopova et al., 2014; Ng et

Table 3.2 Continued

	al., 2014; Beerda et al., 1998; Bauer et al., 2017; Rehn et al., 2017)
Paw lift	One forelimb only is lifted off the ground (Hewison et al., 2014; Ng et al., 2014; Beerda et al., 1998; Van Den Berg et al., 2003)
Pant	Mouth open, tongue exposed with visible, heavy breathing (Protopopova et al., 2014; Stafford et al., 2012; Beerda et al., 1998)
Startle	Sudden, jerking movement of body or head, usually away from a stimulus (Demirbas et al., 2017; Shipper et al., 2008)
Tremble	Visible shaking (shivering) while dog is standing still (Protopopova et al., 2014; Stafford et al., 2012; Beerda et al., 1998)
Freeze	Dog holds body completely still (Bauer et al., 2017)
Catatonic	Dog is completely unresponsive (Bauer et al., 2017)
Escape attempt	Dog attempts to leave pen through the indoor portion (Van Den Berg et al., 2003; Ha & Campion, 2019; Bauer et al., 2017; Firnkies et al., 2017; Rehn et al., 2017)
Frantic	Overly excited, rapid movements (Bauer et al., 2017)
DISRUPTIONS	---
[f]Clean	Kennel being cleaned by caretaker by scraping or hosing floors
[f]Feed	Caretaker adding food to feed dispenser
[f]Treatment	Caretaker performing either 2-minute positive interaction or treat-only treatment

Table 3.2 Continued

[f]Other	Caretaker present in/near kennel for other purposes

Table 3.3. Ethogram of behaviors scored for analysis of focal dog behavior during the daily caretaker interaction and approach tests. Behaviors were not mutually exclusive. Behaviors were scored continuously for entire CI or TO period. All behaviors were scored for duration unless notated with a *. Behaviors notated with a * were scored for frequency. Behaviors notated with a + were not scored during the treat-only (TO) interaction. Behaviors notated with a ^ were not scored during the 1-minute reach of the RYG+ approach test.

BEHAVIOR	DESCRIPTION
LATENCY TO APPROACH[+]	Time taken from opening of pen door to when dog reaches 30 cm from caretaker/experimenter
Near caretaker/experimenter	While oriented toward caretaker/experimenter (Prato-Previde et al., 2003), dog reaches within 30 cm of caretaker/experimenter and remains for minimum of 3 seconds (Topal et al., 2005)
Far from caretaker/experimenter	Dog reaches >30cm from the caretaker/experimenter and remains for a minimum of 3 seconds (Topal et al., 2005)
Move toward caretaker/experimenter	Dog decreases distance between self and caretaker/experimenter (approach) (Protopopova et al., 2014; Hewison et al., 2014; Bauer et al., 2017)
Approach/avoid	Dog alternatively approaches and avoids caretaker/experimenter in rapid succession, not remaining near or far from caretaker/experimenter (Bauer et al., 2017; Finkies et al., 2017) for more than 3 seconds (Topal et al., 2005)
Move away from caretaker/experimenter	Dog increases distance between self and caretaker/experimenter (avoidance)

Table 3.3 Continued

	(Protopopova et al., 2014; Hewison et al., 2014; Van Den Berg et al., 2003; Bauer et al., 2017)
Stationary	Dog is idle and does not approach or avoid caretaker/experimenter
Face caretaker/experimenter	Head oriented so caretaker/experimenter is able to see more than side profile of dog's face (Protopopova et al., 2014)
Face away from caretaker/experimenter	Head oriented so caretaker/experimenter cannot see more than side profile of dog's face
Explore caretaker/experimenter +	Sniff, investigate, lick caretaker/experimenter (Protopopova et al., 2014; Hewison et al., 2014; Ng et al., 2014)
Solicit attention	Dog attempts to gain caretaker's/experimenter's attention (i.e. jumping on pen door/caretaker/experimenter, paw at, rub on, lean on caretaker/experimenter) while oriented toward caretaker/experimenter (Bauer et al., 2017)
Allow contact*+	Dog is within reach of the caretaker/experimenter and allows physical contact (i.e., does not move away)
Play bow	Front legs lie down while hind legs remain standing so that anterior is lowered while posterior is raised. Oriented toward caretaker/experimenter (Protopopova et al., 2014; Bauer et al., 2017)
Belly up	Lying/sitting on ground lifting hind leg or rolling onto back, exposing ventral side;

Table 3.3 Continued

	Oriented toward caretaker/experimenter (Protopopova et al., 2014)
Escape attempt*	Dog attempts to leave pen through the indoor portion (Van Den Berg et al., 2003; Bauer et al., 2017; Firnkies et al., 2017; Rehn et al., 2017)
Wall climbing	Dog attempts to scale wall or pen door
Exit*^	Dog moves from indoor to outdoor portion of pen
Enter*^	Dog moves from outdoor to indoor portion of pen
Destructive oral	Chewing or biting equipment and environment (Demirbas et al., 2017)
Tremble	Visible shaking (shivering) while dog is standing still. (Protopopova et al., 2014; Ng et al., 2014; Beerda et al., 1998; Bauer et al., 2017)
Yawn*	Wide, extended opening of the mouth with deep inhalation of air (Protopopova et al., 2014; Hewison et al., 2014; Beerda et al., 1998; Bauer et al., 2017; Rehn et al., 2017)
Pant	Mouth open, tongue exposed with visible heavy breathing (Protopopova et al., 2014; Ng et al., 2014; Beerda et al., 1998)
Lick lips*	Tongue exits and re-enters mouth in absence of food. (Ng et al., 2014; Beerda et al., 1998; Van Den Berg et al., 2003; Rehn et al., 2017)
Stretch*	Extending body while one or more front/hind legs remain stationary (Protopopova et al., 2014; Ng et al., 2014; Beerda et al., 1998)

Table 3.3 Continued

Shake off*	Rapid, repeated motion of head and/or body back and forth (Protopopova et al., 2014; Ng et al., 2014; Beerda et al., 1998; Bauer et al., 2017; Rehn et al., 2017)
Paw lift	One forelimb only is lifted off the ground (Hewison et al., 2014; Ng et al., 2014; Beerda et al., 1998; Van Den Berg et al., 2003)
Bite threat*	Growl, bare teeth, or snap directed at caretaker/experimenter (McGreevy et al., 2012; Hewison et al., 2014; Van Den Berg et al., 2003; Bauer et al., 2017; Firnkies et al., 2017)
Raised hackles	Hairs on neck, back, and hindquarters rise McGreevy et al., 2012; Van Den Berg et al., 2003; Bauer et al., 2017)
Lunge*	Rapid, aggressive movement toward caretaker/experimenter (Protopopova et al., 2014; McGreevy et al., 2012; Hewison et al., 2014; Ha & Campion, 2019; Bauer et al., 2017)
Repetitive	Dog travels on fixed route (>3 times) i.e. circling, pacing, wall bouncing or other repetitive behavior that is out of context (Bauer et al., 2017)
Frantic	Overly excited, rapid movements (Bauer et al., 2017)
Freeze	Dog holds body completely still (Bauer et al., 2017)
Catatonic	Dog is completely unresponsive (Bauer et al., 2017)

Table 3.3 Continued

Bark*	Short, loud, low frequency vocalization (Protopopova et al., 2014; McGreevy et al., 2012; Ng et al., 2014; Hewison et al., 2014; Beerda et al., 1998; Bauer et al., 2017)
Growl*	Throaty, low, rumbly vocalization (Protopopova et al., 2014; McGreevy et al., 2012; Ng et al., 2014; Hewison et al., 2014; Beerda et al., 1998; Bauer et al., 2017)
Whine*	Cycling, high vocalization (Protopopova et al., 2014; McGreevy et al., 2012; Ng et al., 2014; Beerda et al., 1998)
Howl*	Prolonged high-amplitude vocalization of varying pitch, lips drawn together while exhaling (Protopopova et al., 2014)
TREAT	---
Take treat from hand*^	Dog accepts treat from caretaker directly
Take treat from floor*^	Dog takes treat after caretaker places it on floor
Additional treat*^	Dog takes additional treat not taken by a kennelmate

Table 3.4. Mutually exclusive body language categories based on the body posture and facial expression of focal dogs during caretaker interaction and human approach tests (Bauer et al., 2017). Behaviors were mutually exclusive. Behaviors were scored continuously for duration for entire interaction or test period.

BODY LANGUAGE	DESCRIPTION
Fearful (red)	Eyes wide open with pupils dilated and sclera visible, scanning/darting, slow blinking, or hard staring; ears pulled back caudally and distally, or pulled high, tense or with wrinkles between them; closed mouth with tense muzzle and wrinkles in brow, teeth may be visible; tail low and still or wagging, tucked, mid-way up

Table 3.4 Continued

	and still, high up over back with or without flagging; tense or hard musculature with uneven body weight, either distributed-forward, -backwards, or –laterally (e.g. low and back, forward and hard)
Non-fearful (green)	Neutral ears and eyes; tail_neutral or wagging, midway or high up; mouth open and relaxed with no visible tension in face, muzzle, and brow; soft or relaxed musculature with body weight evenly distributed
Ambivalent (yellow)	Mix of fearful and non-fearful body language and postures, cannot clearly categorize as red or green

3.3 Data Analysis

All analyses were conducted using Microsoft Excel and R Studio (RStudio Team, 2020). Preliminary, descriptive statistics including means, standard deviations of the means, proportions, chi-square tests, histograms, scatterplots, and boxplots were conducted to guide further analyses. Criteria for statistical significance was set at $\alpha < 0.05$ unless otherwise specified. Facilities were analyzed separately due to differences in management, style of caretaker interaction, and small facility sample size. Each focal dog served as its own control.

3.3.1 Inter-Rater Reliability

Four different experimenters assigned live scores of red, yellow, and green (RYG) to dogs during the RYG approach only (when focal dogs were free to exit the indoor portion of the pen) and during the three steps of the behavioral portion of the Field Instantaneous Dog Observation tool (RYG+). Prior to live scoring of focal dogs, the four experimenters assigned RYG scores to dogs from video recordings of the RYG+ approach test. Interclass correlation coefficients (ICCk3s) with 95% confidence intervals were utilized to assess inter-rater reliability of RYG scores between experimenters conducting live approach tests and behavioral scoring from video (Ko & Li, 2016). For all video behavioral scoring done by a set of independent scorers, a sample of 72 videos (17% of all videos) were scored by each individual for reliability. According to Koo and Li (2016), an ICC score of below 0.50 is considered poor, scores greater than 0.50 and up to 0.75 are considered fair, scores greater than 0.75 and up to 0.90 are considered good, and a score above 0.90 is considered excellent (Koo & Li, 2016).

3.3.2 Physical Metrics

Descriptive statistics were used to determine prevalence of differing body condition and cleanliness scores, along with presence of all other health metrics in the physical portion of the FIDO test. Each physical metric was the outcome variable in each model with treatment and timepoint as fixed effects, and dog ID nested within pen ID as random effects, using the "REML" method. Residuals from the models were tested for normality with a histogram and scatterplot. If residuals were not normal, normality was increased with a log-transformation, although general linear mixed effects models have been shown to be robust to non-normal data (Schielzeth et al., 2020). Models were assessed in a backwards stepwise manner, meaning interactions followed by variables (treatment and day) were sequentially eliminated using "AIC" values and "ML" to determine the best fit. Interactions and variables were removed from a model if they did not lower the "AIC" score. The best fitting model was then run with "REML," and test statistics were determined using Wald's test.

3.3.3 Physiological Metrics

Hair cortisol concentration (HCC) was analyzed using paired t-tests, Wilcoxon signed rank tests, and generalized linear mixed effects models (GLMEs). For paired t tests, normality was tested via Shapiro-Wilk tests. If data were not normal, they were log-transformed. If normality was achieved after transformation, the log-transformed data were analyzed with paired t tests. If normality was not achieved, data were analyzed using Wilcoxon signed rank tests. Paired t tests were used to compare HCC values between Days 0 and 28 within each treatment group (A or B) and for all dogs at the facility combined. GLMEs were run using the methodology described above with sIgA or HCC as outcome variables, timepoint and treatment type as fixed effects, and dog ID nested within pen ID as random effects.

3.3.4 Behavioral Metrics

Descriptive statistics were used to determine proportions of RYG scores for each facility, treatment group, and time point. RYG scores for each step of the approach tests at each time point were analyzed using generalized linear mixed effects models (GLMEs). GLMEs were run using the methodology described above, with RYG score at each step as the outcome variable, tester (i.e., unfamiliar experimenter or familiar caretaker), treatment, and time point as fixed effects, and dog ID nested within pen ID as random effects. Additionally, RYG scores at each step were added to create a sum RYG score, which was the outcome variable in a model with the same fixed and random effects. Finally, frequencies and durations of behaviors from the time surrounding the caretaker interaction, caretaker interaction itself, and the one-minute reach portion of the RYG+ approach test ethograms were analyzed using the same methodology. Tester was not used as a fixed effect for models involving the caretaker interaction as there was no unfamiliar experimenter involved.

3.3.5 Associations Between Metrics

To determine whether any of the variables influenced each other, a correlation matrix was used with all metrics collected at similar timepoints. For the behavioral metrics that were not collected on Days 0, 14, and 28 (e.g., behavior during the caretaker interaction), multiple regression models were run with HCC, sIgA, RYG score, or behavior during the 1-minute reach of the RYG+ at each individual timepoint as the outcome variable and behavior surrounding the caretaker interaction or during the caretaker interaction as predictors with dog ID nested within pen ID as random effects. For example, RYG score at Day 14 was an outcome variable with each behavior during the caretaker interaction at each timepoint as predictors.

CHAPTER 4. RESULTS

4.1 Facility 1

4.1.1 Physical Health and Physiological Metrics

Throughout the study, the majority of dogs in Facility 1 appeared clean and healthy. Physical health metrics from the FIDO tool observed at this facility included body cleanliness scores of 0% and 1-25%; ocular discharge; mild, moderate, and severe tear staining; coughing; missing fur and poor coat; and healing wounds (see Figure 4.1).

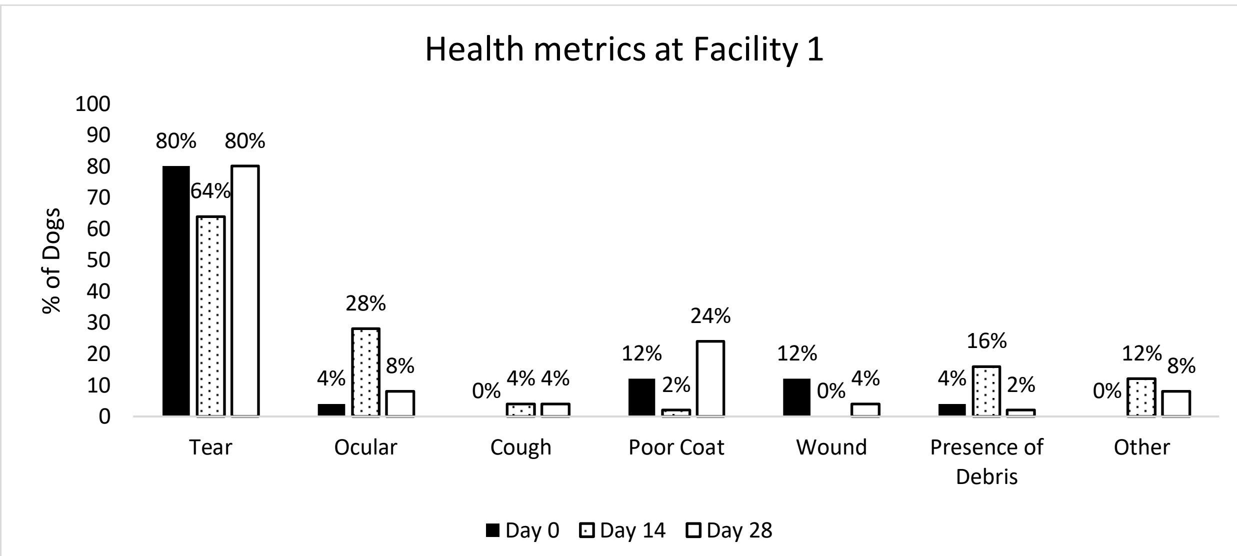


Figure 4.1. Health metrics from the Field Instantaneous Dog Observation Tool (FIDO) observed at Facility 1. Metrics observed include tear staining, ocular discharge, coughing, poor coat, healed wounds, and presence of debris on the dog's body.

Fecal sIgA decreased significantly over time in Facility 1 (X^2=21.117, p<0.001). A post-hoc Tukey test revealed significant differences between Days 0 and 14 (p=0.001) and Days 0 and 28 (p=0.010). When examining treatment groups separately, there were significant changes in both the CI group (2-minute positive interaction with treats) and the TO group (treat-only). Fecal sIgA in the CI group decreased (X^2=12.352, p=0.002), with a post-hoc Tukey test showing a significant decrease from Day 0 to Day 14 (p=0.019). There was a subsequent increase in sIgA from Day 14 to Day 28, although it was not significant. Similarly, sIgA in the TO group decreased significantly (X^2=10.750, p=0.005), with Days 0 and 28 being significantly different (p=0.046) (see Figure 4.2).

Mean sIgA over time at Facility 1

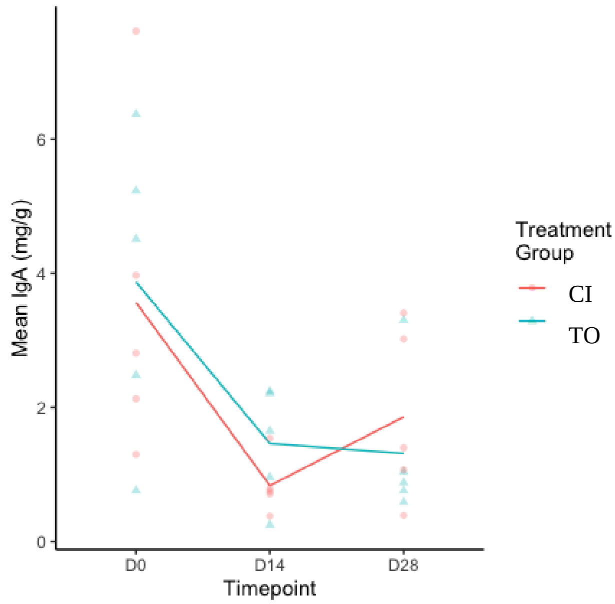


Figure 4.2. Fecal sIgA over time at Facility 1 with individual data points. Fecal sIgA decreased significantly over time in the CI group (X^2=12.352, p=0.002) from Day 0 to 14 (p=0.019) and the TO group (X^2=10.750, p=0.005) from Day 0 to 28 (p=0.046).

After removing outliers from the analysis, hair cortisol concentration (HCC) increased significantly between Days 0 and 28 (X^2=6.270, p=0.012, Day 0: n=23, Day 28: n=20), with no effect of treatment type (see Figure 4.3). When examining the two treatment groups separately, it was found that only the CI group showed a significant increase in HCC (X^2=10.249, p=0.001). See Figure 4.4 for a summary of changes in physiological health metrics.

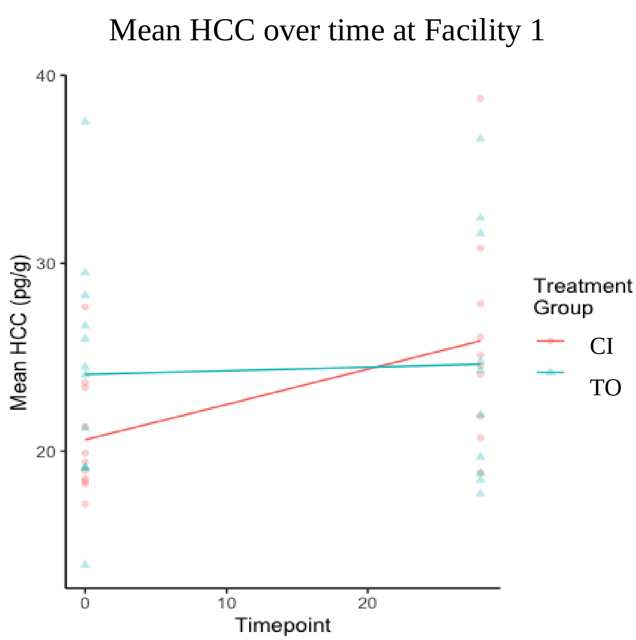


Figure 4.3. HCC over time at Facility 1 with individual data points. HCC significantly increased over time (X^2=6.270, p=0.012) in both treatment groups combined. HCC significantly increased in the CI group (X2=10.249, p=0.001), but not in the TO group alone.

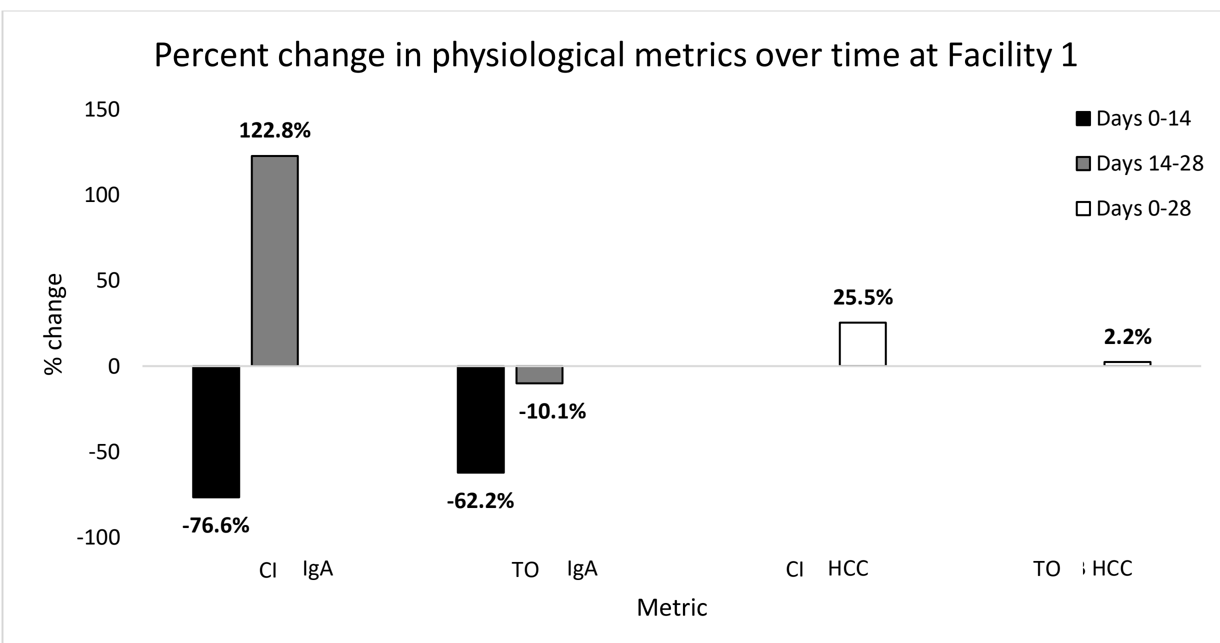


Figure 4.4. Mean percent change in physiological metrics between Days 0 and 14, Days 14 and 28, and Days 0 and 28 (HCC only) at Facility 1.

4.1.2 Inter-Rater Reliability

All stages of the RYG+ resulted in inter-class correlation (ICC) estimates of inter-rater agreement of over 0.9, indicating excellent agreement according to Koo and Li (2016) (see Table 4.1).

Table 4.1. ICC estimates of inter-rater reliability between 4 raters of RYG scores during the FIDO+ approach test

Step of RYG+	**ICC Estimate**
Approach (RYG)	0.994
Open (RYG)	0.949
Reach (RYG)	0.968
Touch (yes or no)	0.904

Five independent raters scored behavior from video in the four hours surrounding the daily interaction. Agreement of mutually exclusive categories (area of pen [i.e., indoor or outdoor portion, or between], location in pen [i.e., front or back], proximity to kennel mate, posture, and activity [i.e., idle, slow locomotion, or rapid locomotion]) were calculated using Light's Kappa and resulted in values ranging from 0.724 (moderate agreement) to 0.908 (near perfect agreement) (see Table 4.2) (McHugh, 2012). Non-mutually exclusive behaviors ranged in ICC estimates from 0.638 (moderate agreement) to 0.991 (excellent agreement) (Koo & Li, 2016). Not all behaviors in the ethogram (Table 3.2) occurred with enough frequency to calculate reliability (Table 4.3).

Table 4.2. Kappa estimates of inter-rater reliability between five independent raters of mutually exclusive behavioral categories scored from video in the four hours surrounding the daily caretaker interaction.

Category	**Kappa (*K*)**
Area	0.724
Location	0.908
Proximity to kennel mate	0.836
Posture	0.870
Movement	0.776

Table 4.3. ICC estimates of inter-rater reliability between five independent raters of non-mutually exclusive behaviors occurring with sufficient frequency scored from video in the four hours surrounding the daily caretaker interaction.

Behavior	**ICC Estimate**
Vigilant	0.853
Rest	0.991
Explore	0.886
Groom	0.638
Interact with enrichment	0.978
Eat	0.898
Repetitive	0.909
Enter	0.857
Exit	0.785
Jump on pen door	0.756

4.1.3 Body Language in Response to Human Approach

Human approach tests

There was no significant change in response to stranger approach to the indoor portion of the pen during the RYG approach only at Facility 1. No significant changes in sum RYG+ scores were observed over time, in response to different testers (i.e., familiar caretaker or unfamiliar experimenter), or according to treatment group. However, when examining the steps of the RYG+ separately, timepoint significantly affected RYG score in response to the "reach" step of the RYG+ (X^2=10.549, p=0.001). Post-hoc testing revealed significant differences between Days 0 and 14 (p=0.017) and Days 0 and 28 (p=0.004), in which dogs from both groups combined showed higher RYG scores on Days 14 and 28 than on Day 0. Further analysis revealed that RYG scores increased significantly over time in the TO group (X^2=7.717, p=0.021) only, with the difference between Days 0 and 28 being significant (p=0.020) (See Figures 4.5-4.8).

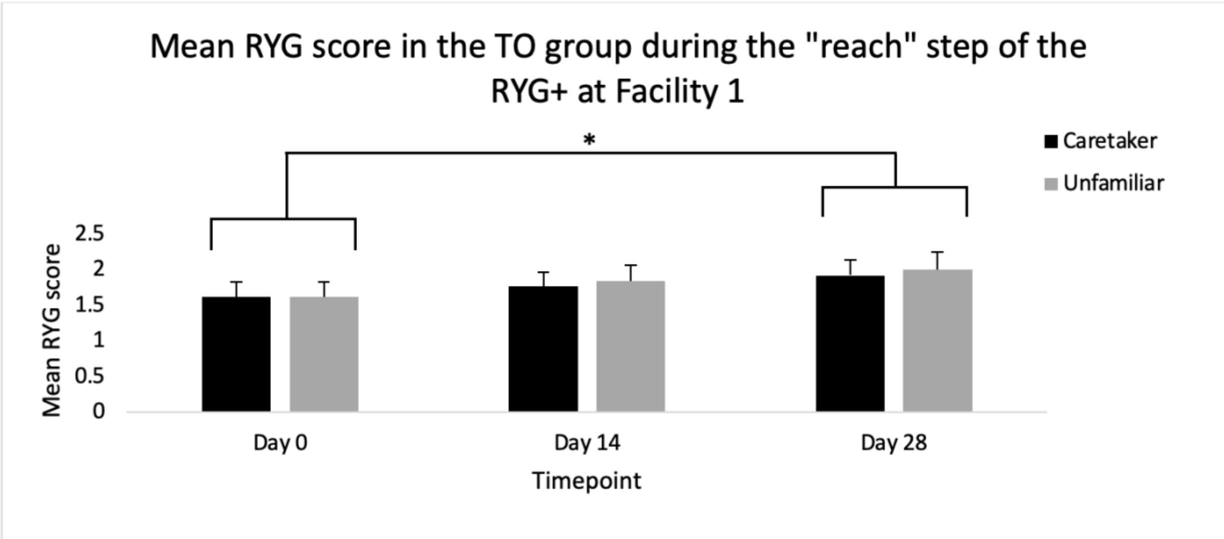


Figure 4.5. Mean RYG scores during the “reach” step of the RYG+ over time in the TO group at Facility 1. Numerical scores were assigned to scores of red (1), yellow (2), and green (3), so that an increase in RYG score indicates less fearful behavior over time. RYG scores in TO dogs were higher on Day 28 than Day 0 (p=0.020), with no effect of tester (familiar caretaker or unfamiliar experimenter). * Denotes p<0.05. Error bars represent standard error of the mean.

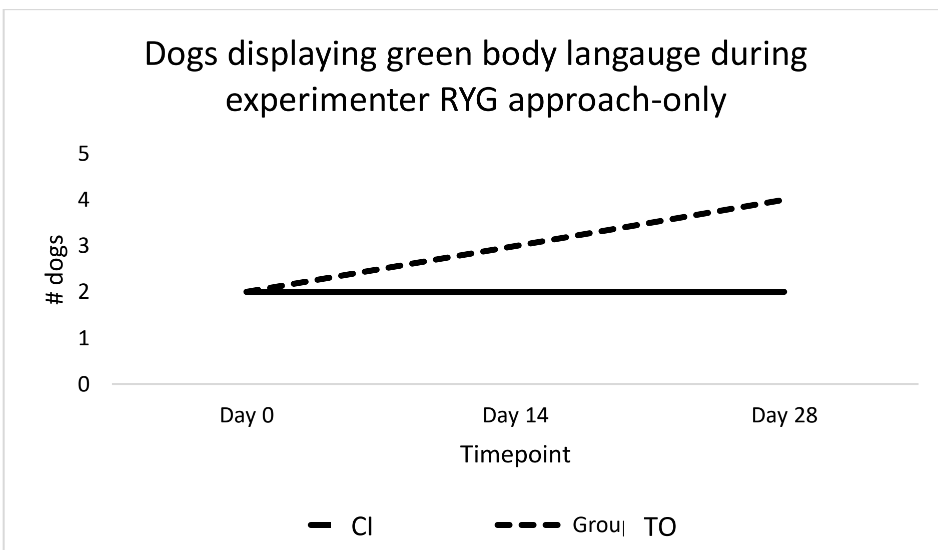


Figure 4.6. Descriptive representation of the number of dogs at Facility 1 displaying green (non-fearful) body language during the unfamiliar experimenter RYG approach-only in which dogs were free to exit to the outdoor portion of their pens. There were no significant changes in RYG scores over time.

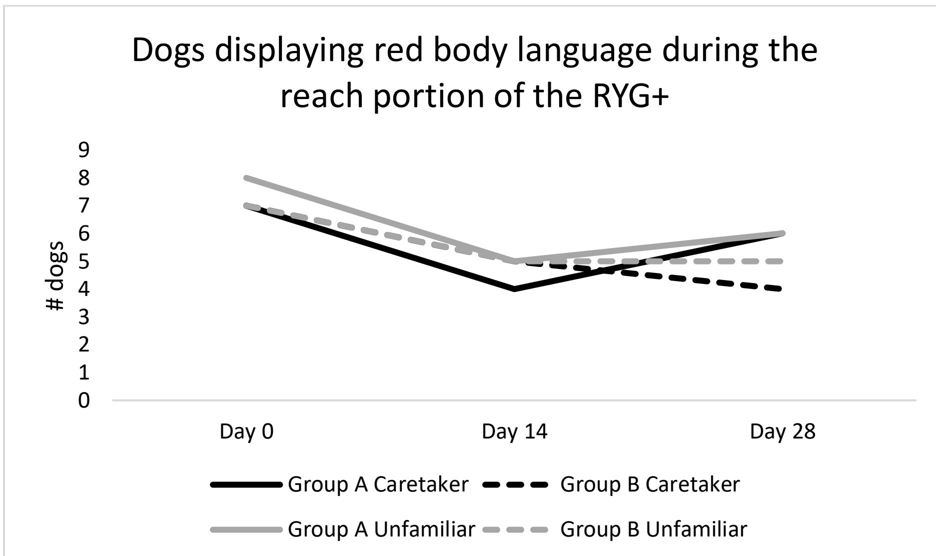


Figure 4.7. Descriptive representation of the number of dogs at Facility 1 displaying red (fearful) body language during the "reach" portion of the RYG+. A decrease in red body language indicates less fearful body language over time. Treatment type and tester (i.e., familiar caretaker or unfamiliar experimenter) did not have an effect, however mean RYG scores significantly changed over time (X^2=10.549, p=0.001), with a significant increase from Day 0 to 28 (p=0.020) in the TO group, indicating a decrease in fearful body language.

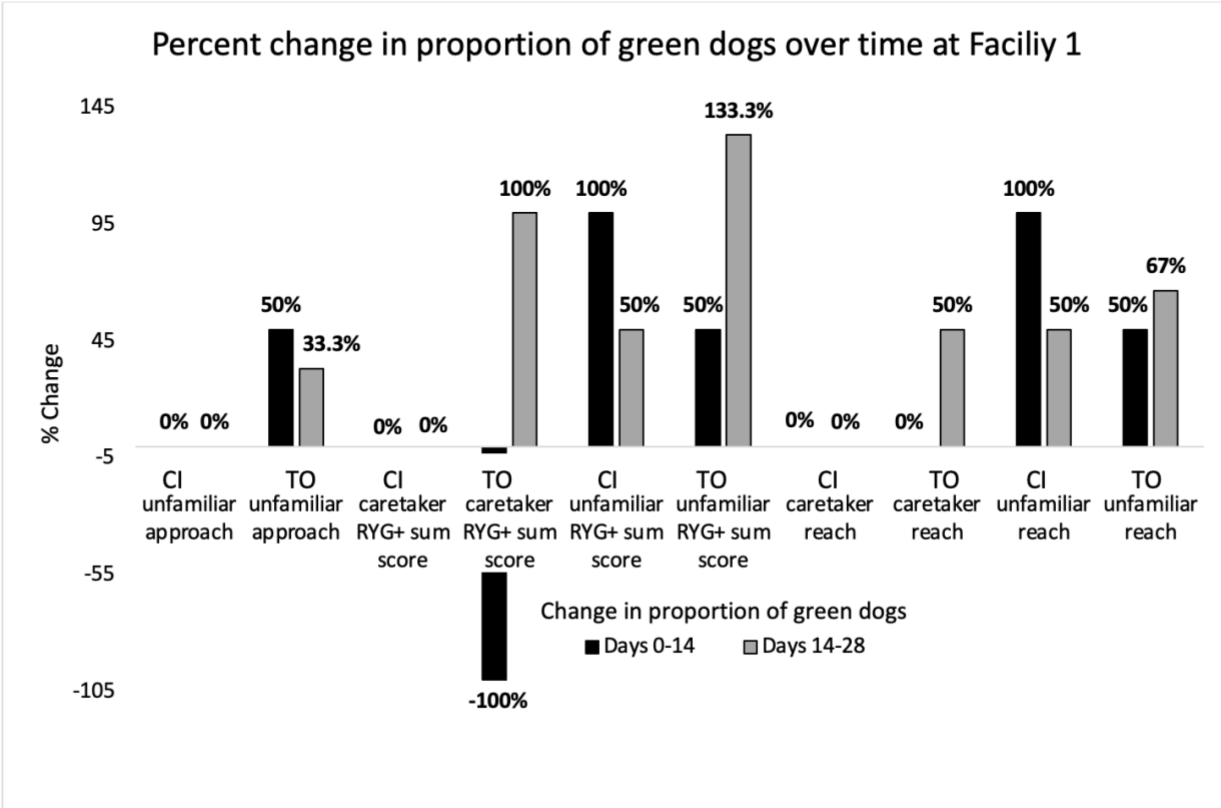


Figure 4.8. Percent change in proportion of dogs showing non-fearful (green) body language during human approach tests between Days 0 and 14, and Days 14 and 28 at Facility 1.

Daily caretaker interaction

During the 2-minute caretaker interaction (CI), time spent displaying green body language increased (X^2=11.732, p=0.001) and yellow body language decreased (X^2=9.668, p=0.002) (Figure 4.9) over time. In the TO group, however, body language during the caretaker interaction did not change.

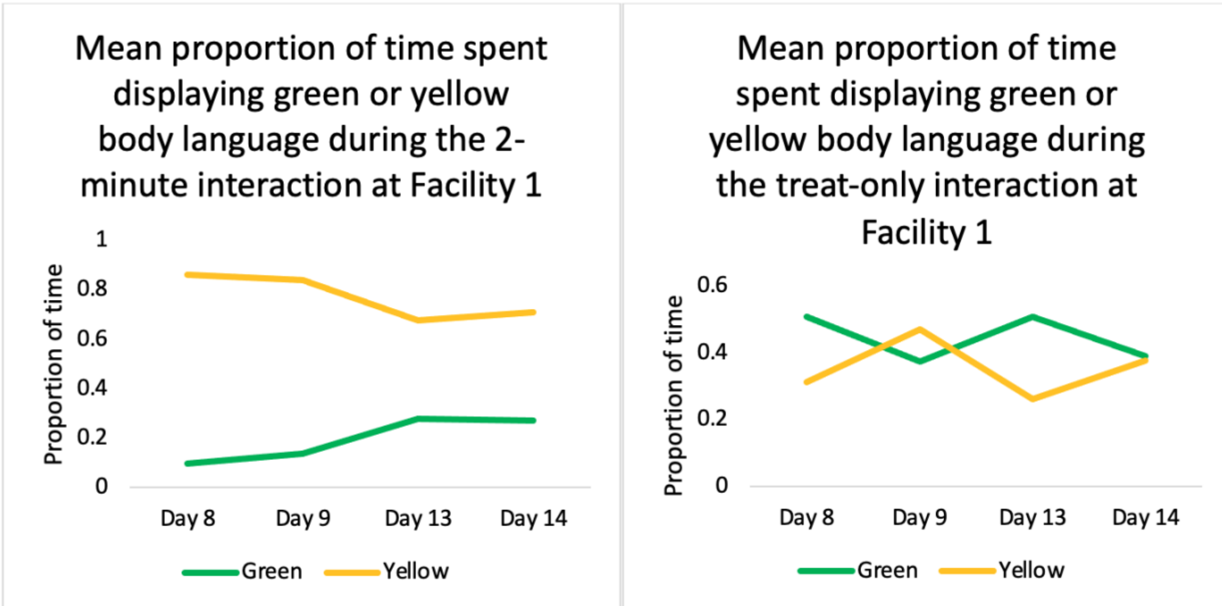


Figure 4.9. Proportion of time spent displaying non-fearful (green) and ambivalent (yellow) body language during the daily 2-minute and treat-only interactions at Facility 1. Time spent displaying green behavior significantly increased (X^2=11.732, p=0.001) and time spent displaying yellow behavior significantly decreased (X^2=9.668, p=0.002) over time in the CI group. No significant changes were observed in the TO group.

4.1.4 Latency to Approach

Due to malfunction of the security camera system, video recording at Facility 1 was lost on Days 0, 1, and 2. Therefore, all analyses on behavior scored from video only examine FIDO+ testing on Days 14 and 28. Latency to approach the tester did not change significantly over time or as a function of treatment group or tester. However, it is interesting to note that latency to approach was shorter on Day 28 than Day 14 in all tests except for the CI group during the caretaker RYG+ (see Figure 4.10).

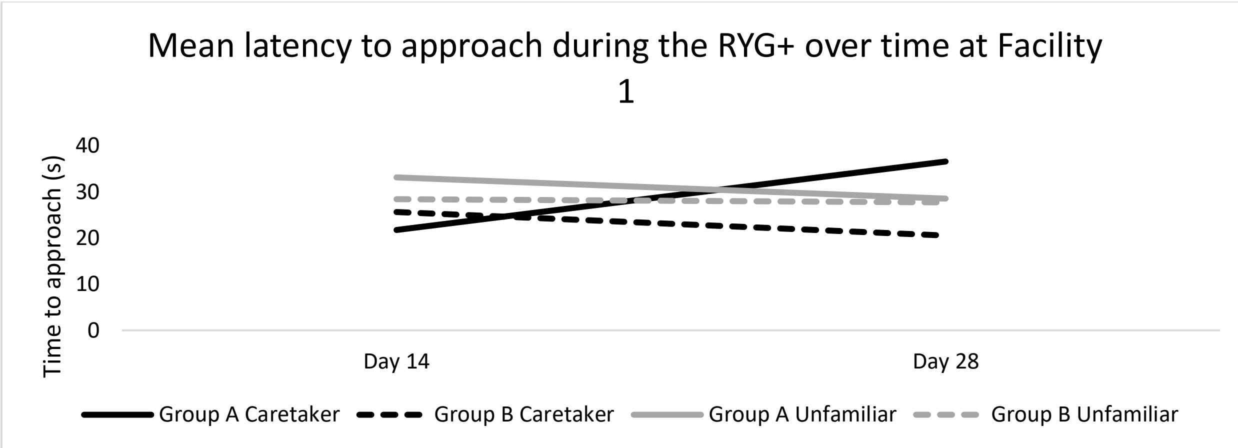


Figure 4.10. Mean latency to approach tester during the 1-minute reach portion of the RYG+ approach test over time at Facility 1. No significant change was observed in the time it took dogs to approach the tester.

4.1.5 Interaction with Caretaker and Unfamiliar Experimenter

Human approach tests

Behaviors during the 1-minute reach portion of the RYG+ were scored from an ethogram (Table 3.3). Behavior was scored for duration and analyzed as the proportion of time during the 1-minute reach portion of the RYG+ spent performing each behavior. Again, all analyses on behavior scored from video during the human approach tests only examine RYG+ testing on Days 14 and 28. In the CI group, frequency of lip licking decreased (X^2=7.782, p=0.005), duration of vigilance (i.e., watching the tester) increased (X^2=6.610, p=0.010), and moving away from the tester decreased (X^2=5.413, p=0.020) significantly over time (see Figures 4.11 and 4.12). Additionally, more lip licks were observed (X^2=4.631, p=0.031) and dogs spent more time moving away from the caretaker than the unfamiliar experimenter (X^2=5.413, p=0.020) during this test.

In the TO group, time spent oriented toward the tester increased (X^2=4.154, p=0.042) and time spent vigilant increased (X^2=4.396, p=0.036) over time. Additionally, proportion of time spent oriented toward the tester was higher during the unfamiliar experimenter reach than the familiar caretaker reach (X^2=5.041, p=0.025) (see Figure 4.13).

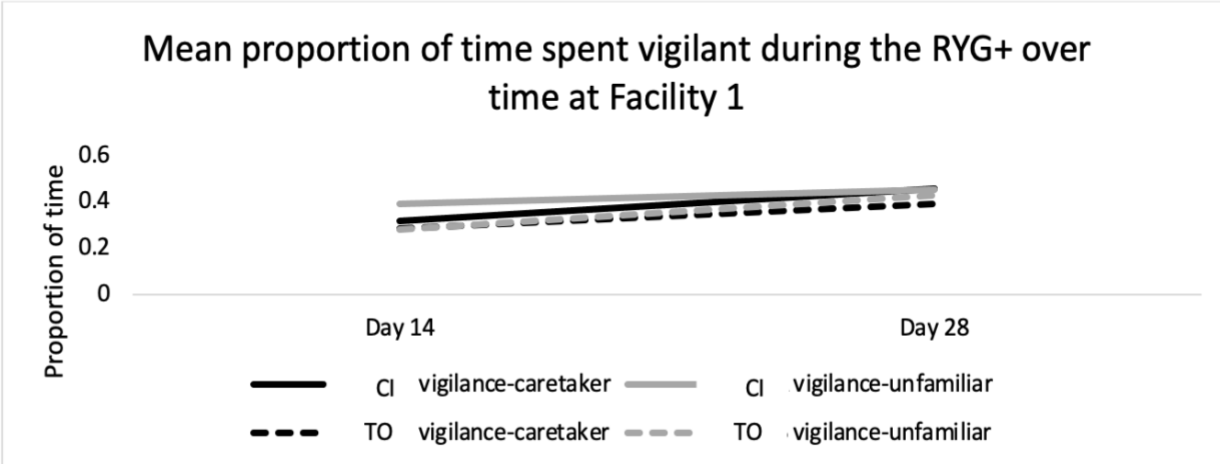


Figure 4.11. Mean proportion of time spent vigilant during the 1-minute reach portion of the RYG+ over time at Facility 1. Vigilance increased over time in the CI group (X^2=6.610, *p*=0.010) and the TO group (X^2=4.396, *p*=0.036).

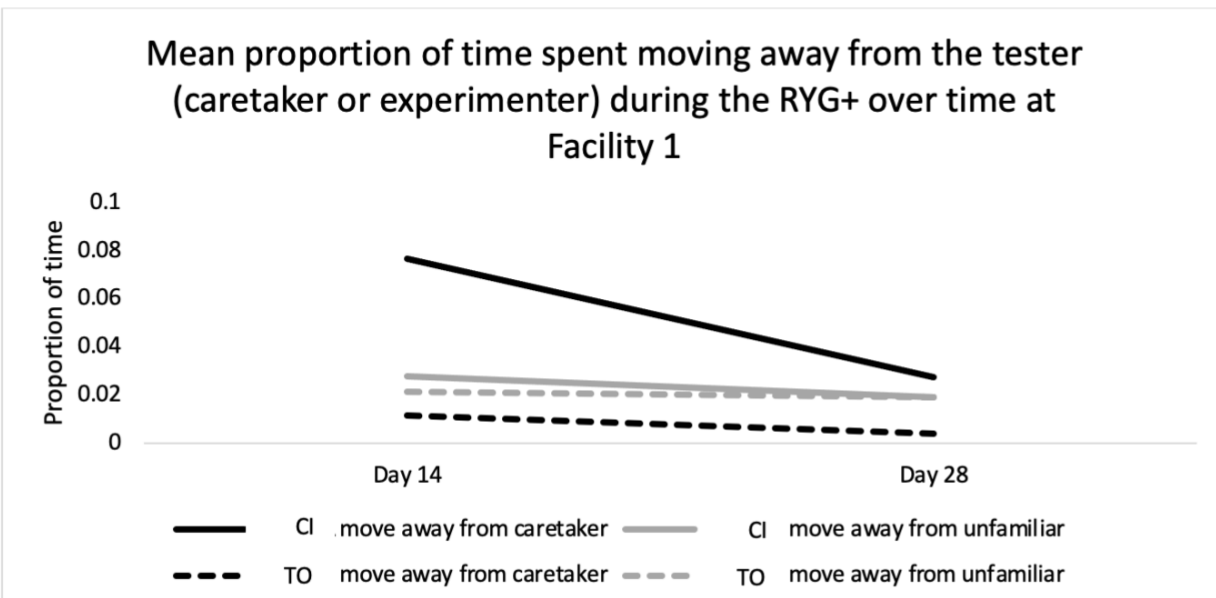


Figure 4.12. Mean proportion of time spent moving away from the tester (familiar caretaker or unfamiliar experimenter) during the 1-minute reach portion of the RYG+ over time at Facility 1. The CI group significantly decreased in time spent moving away from the tester over time (X^2=5.413, *p*=0.020).

Daily caretaker interaction

Behaviors during the daily caretaker interaction were scored from video using an ethogram (Table 3.3) on Days 1, 2, 8, 9, 13, and 14. Again, due to video loss, only Days 8, 9, 13, and 14 were scored. Time spent in proximity (≤1 meter) to the caretaker increased significantly over time in both treatment groups (CI: X^2=14.047, *p*=0.000; TO: X^2=5.121, *p*=0.024). Proximity to the caretaker in the CI group was scored for duration and measured as a proportion of the total interaction time spent in that behavior. In the TO group, due to the rapid nature of the treatment, proximity to the caretaker was scored as frequency and analyzed as the rate of events per interaction (see Figure 4.14). Additionally, orientation away from the caretaker increased (X^2=4.574, *p*=0.0325), and vigilance decreased (X^2=4.311, *p*=0.038) throughout the treatment.

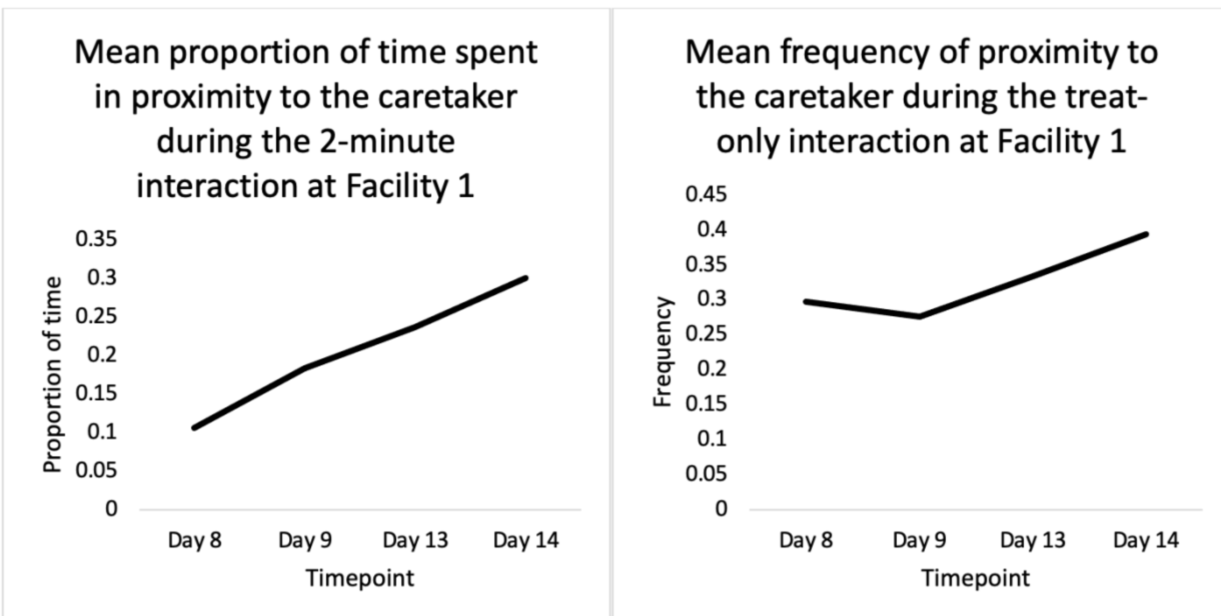


Figure 4.13. Time spent in proximity (≤1 meter) to the caretaker during the daily interaction over time at Facility 1. In the CI group, time spent in proximity to the caretaker significantly increased over time (X^2=14.047, *p*<0.001), and in the TO group, frequency of times dogs chose to be near the caretaker also increased over time (X^2=5.121, *p*=0.024).

4.1.6 Preferred Location in Pen and Locomotion During the Daily Interaction

In the CI group, the area (i.e., indoor or outdoor portion of the pen, or between the indoor and outdoor portion) in which dogs chose to spend time during the interaction changed significantly over time. Time spent indoors increased (X^2=16.032, p=0.000) and time spent outside decreased (X^2=9.735, p=0.002) (Figure 4.15). Additionally, the CI group, locomotion in the indoor portion of the pen increased (X^2=3.989, p=0.046). In the TO group, preferred area of the pen did not change during the daily interaction. However, frequency of exits (e.g., from the indoor to the outdoor portion of the pen) decreased over time (X^2=6.597, p=0.010).

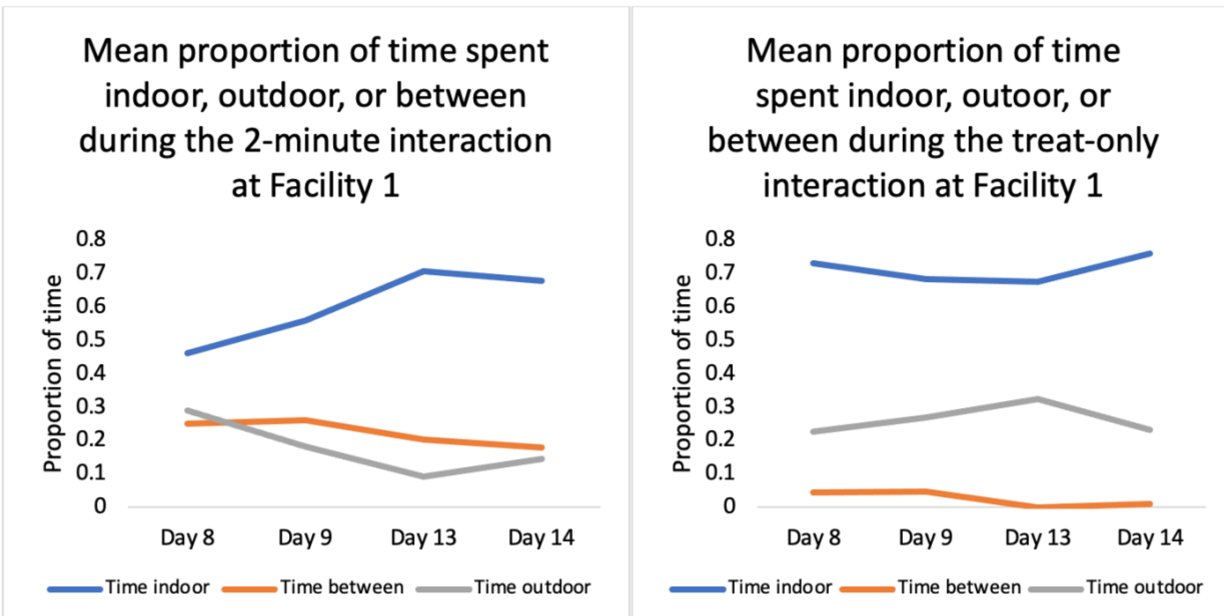
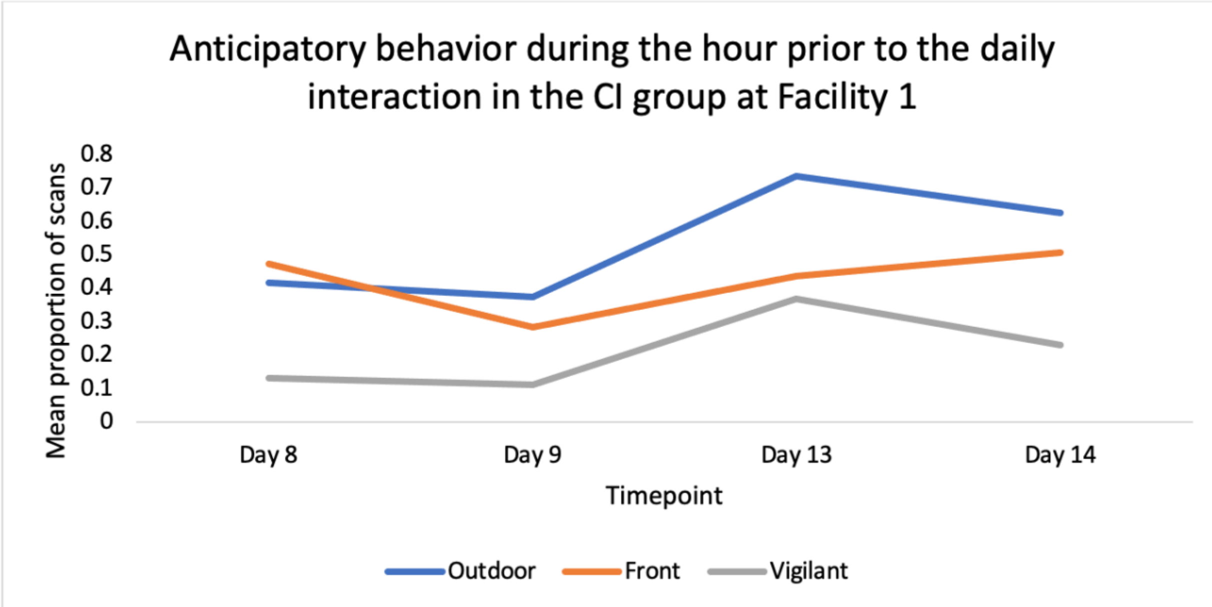


Figure 4.14. Proportion of time spent in the indoor, outdoor, and between portions of the pen during the daily 2-minute interaction and treat-only interactions at Facility 1. Time indoor and outdoor changed significantly over time in the CI group (X^2=16.032, p<0.001; X^2=9.735, p=0.002). No significant change over time in area of the pen occurred in the TO group.

4.1.7 Behavioral Time Budget Preceding the Daily Caretaker Interaction

Behavior starting in the hour prior to the daily interaction and ending with two hours after the interaction was scored from video on Days 1, 2, 8, 9, 13, and 14. Again, due to video loss, only Days 8, 9, 13, and 14 were scored from video using an ethogram (Table 3.3). Behavior was analyzed as proportion of scans per hour in which it was observed. Hour and treatment type were not found to significantly affect behavior over time; however, certain behaviors did change over multiple days, regardless of hour or treatment type. In the hour prior to the interaction, time spent outdoors increased (X^2=6.675, p<0.001), vigilance increased (X^2=4.118, p=0.042), and self-grooming decreased (X^2=4.195, p=0.041) over time. In addition to anticipatory behaviors, such as vigilance and time spent outdoors (where dogs in this facility could see the caretaker approaching the building), time spent at the front of the pen increased over time (Figure 4.16).



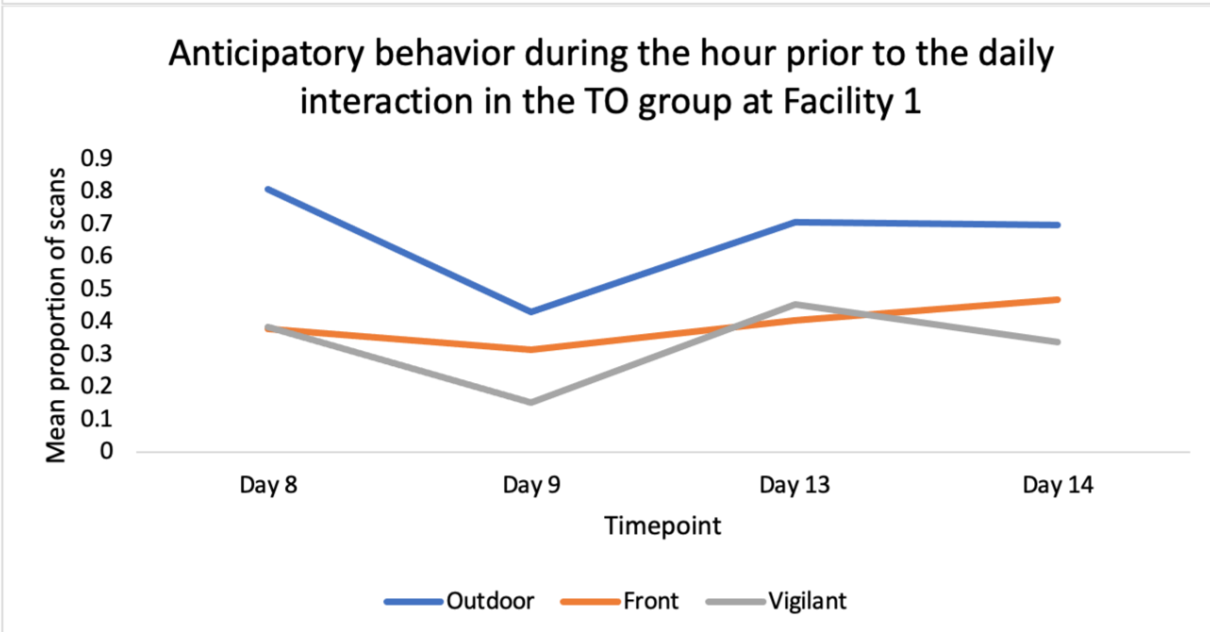


Figure 4.15. Anticipatory behavior (outdoor area of the pen, front of the pen, and vigilance) in the hour prior to the daily caretaker interaction measured as a proportion of scans/hour in which focal dogs displayed each behavior. Timepoint had a significant effect on vigilance in the TO group (X^2=7.817, *p*=0.005), although the direction of change fluctuated and time spent outdoors increased in both treatment groups (X^2=6.675, *p*=0.000).

4.2 Facility 2

4.2.1 Physical Health and Physiological Metrics

Like Facility 1, the majority of dogs at Facility 2 appeared clean and healthy. Physical health metrics from the FIDO tool observed at this facility include body cleanliness scores of 0% and 1-25%; ocular discharge; mild, moderate, and severe tear staining; missing fur or poor coat; healing wounds, and matting (see Figure 4.17).

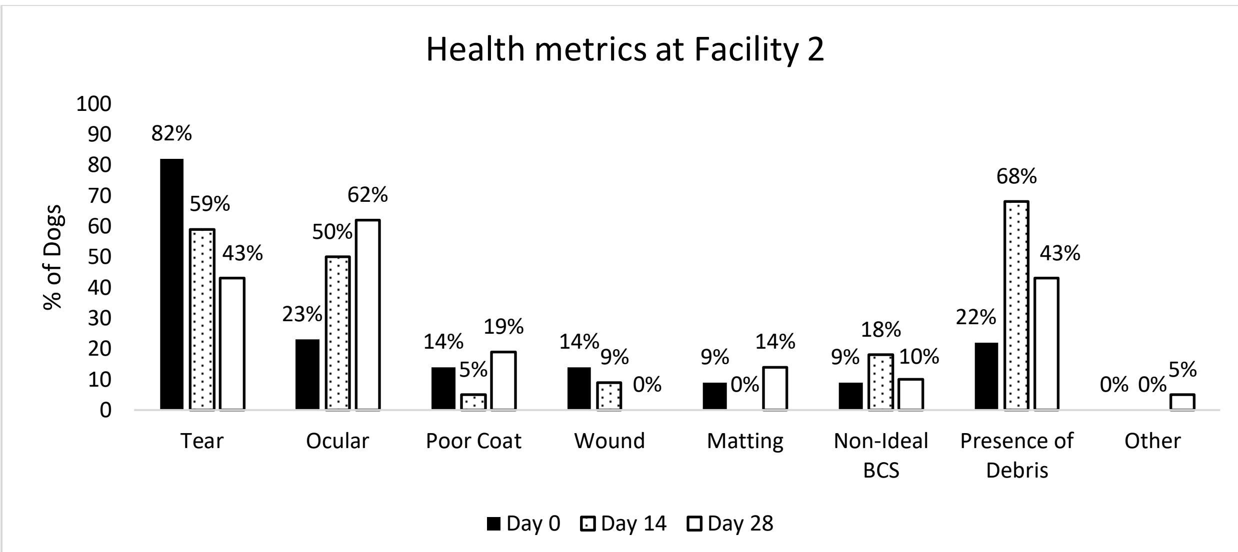


Figure 4.16. Health metrics from the Field Instantaneous Dog Observation Tool (FIDO) observed at Facility 2. Metrics observed include tear staining, ocular discharge, missing fur or poor coat, healed wounds, matted fur, over- or under-weight body condition score, and presence of debris on the dog's body.

There was no significant change in sIgA at Facility 2 over time or according to treatment type (Figure 4.18). HCC decreased significantly (X^2=6.661, p=0.010) again, with no effect of treatment type (see Figure 4.19). Neither treatment group showed significant changes in HCC when analyzed separately. Additionally, a mixed effects model revealed HCC to significantly affect fecal sIgA (X^2=4.099, p=0.043). See Figure 4.20 for a summary of changes in physiological health metrics.

Mean sIgA over time at Facility 2

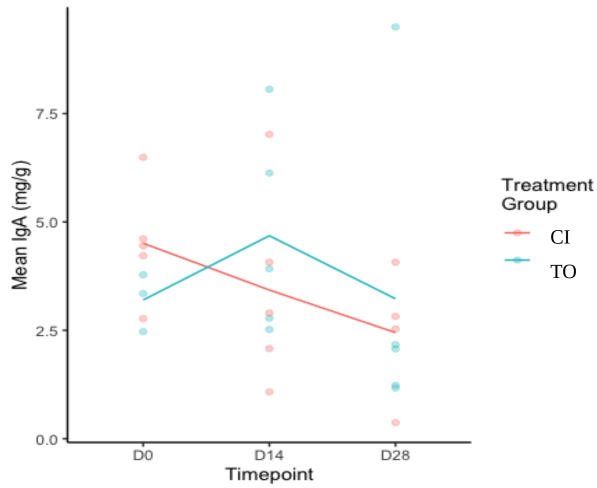


Figure 4.17. Fecal sIgA over time at Facility 2 with individual data points. One outlier from the CI group was removed at each timepoint. No significant changes occurred over time or between treatment groups.

Mean HCC over time at Facility 2

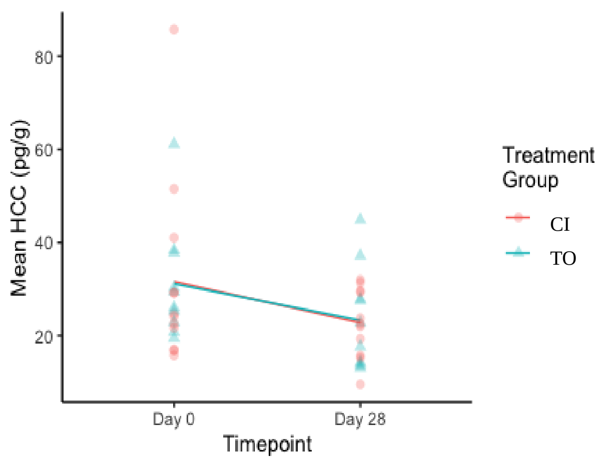


Figure 4.18. HCC at Facility 2 with individual data points. HCC significantly decreased in combined treatment groups only (X^2=6.661, p=0.010).

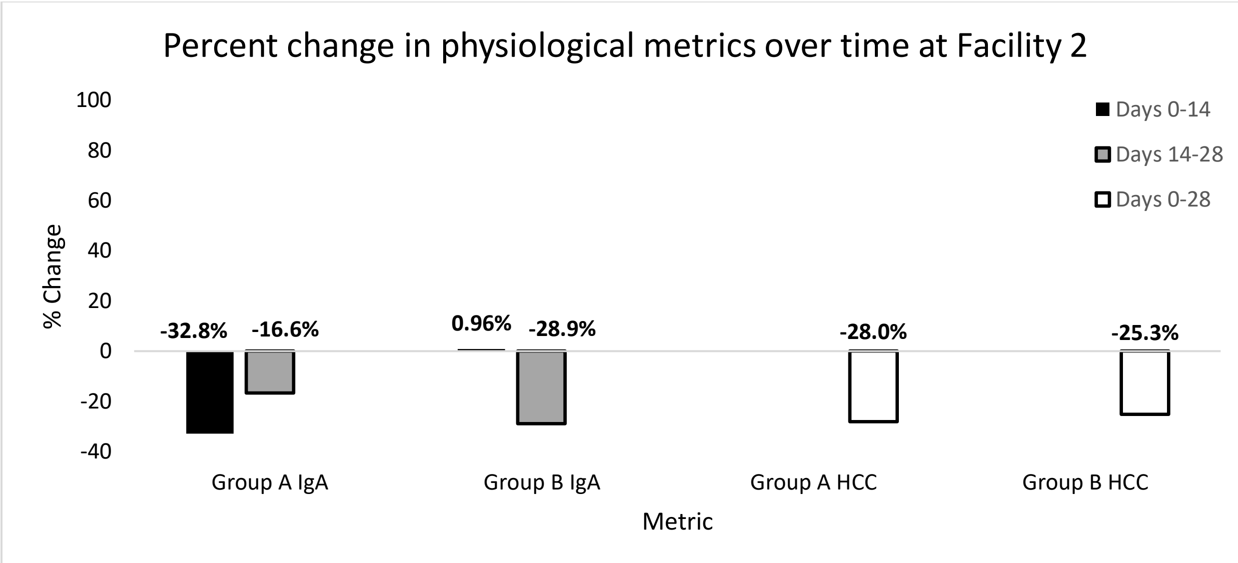


Figure 4.19. Mean percent change in physiological metrics between Days 0 and 14, Days 14 and 28, and Days 0 and 28 (HCC only) at Facility 2.

4.2.2 Body Language in Response to Human Approach

Human approach tests

The same inter-rater reliability as described for Facility 1 above was applied to Facility 2. RYG scores changed significantly with an interaction between treatment type and timepoint (X^2=12.451, p=0.002) during the RYG unfamiliar experimenter approach-only (Figure 4.21). No significant differences between timepoints were found with a post-hoc Tukey test. However, when examining treatment groups separately, significant differences between timepoints were revealed. In the CI group, RYG score decreased over time (X^2=8.519, p=0.014), with Days 0 and 28 differing significantly (p=0.041). Again, in the TO group, RYG scores increased over time (X^2=7.571, p=0.023), with a significant difference between Days 0 and 14 (p=0.044).

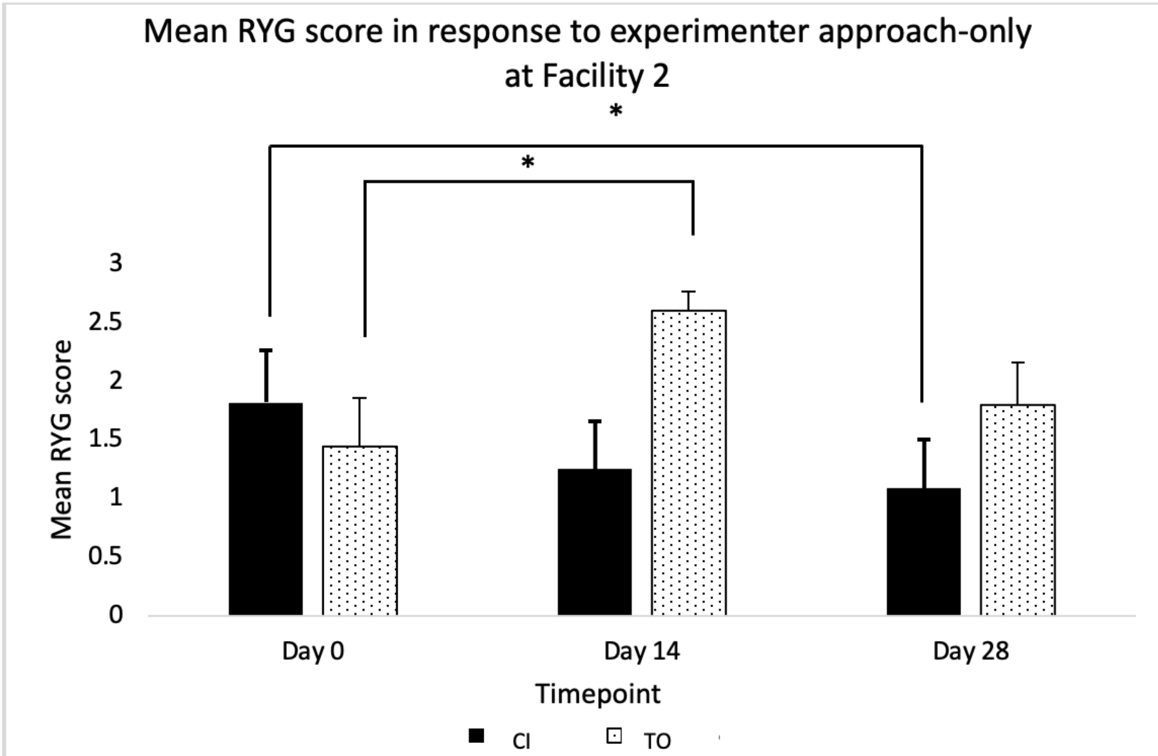


Figure 4.20. Mean RYG score in response to approach of an unfamiliar experimenter during the RYG-only approach test at Facility 2. Numerical scores were assigned to scores of red (1), yellow (2) and green (3) so that an increase in RYG score indicates less fearful behavior over time. RYG score increased in the CI group from Day 0 to Day 28 (p=0.041) and in the TO group from Day 0 to Day 14 (p=0.044). * Denotes $p<0.05$. Error bars represent the standard error of the mean.

There were no significant changes in the sum RYG+ approach test score (Figure 4.22). However, RYG scores during the "reach" step of the RYG+ significantly increased over time (X^2=13.5782, p=0.001) (see Figure 4.23). Timepoint had a significant effect on the CI group's mean RYG response to the caretaker's (X^2=4.305, p=0.037) and unfamiliar experimenter's (X^2=5.036, p=0.025) reach, while timepoint had a significant effect on the TO group's response to the unfamiliar experimenter's reach only (X^2=5.816, p=0.016). Post-hoc testing revealed no significant differences between timepoints (Figure 4.24).

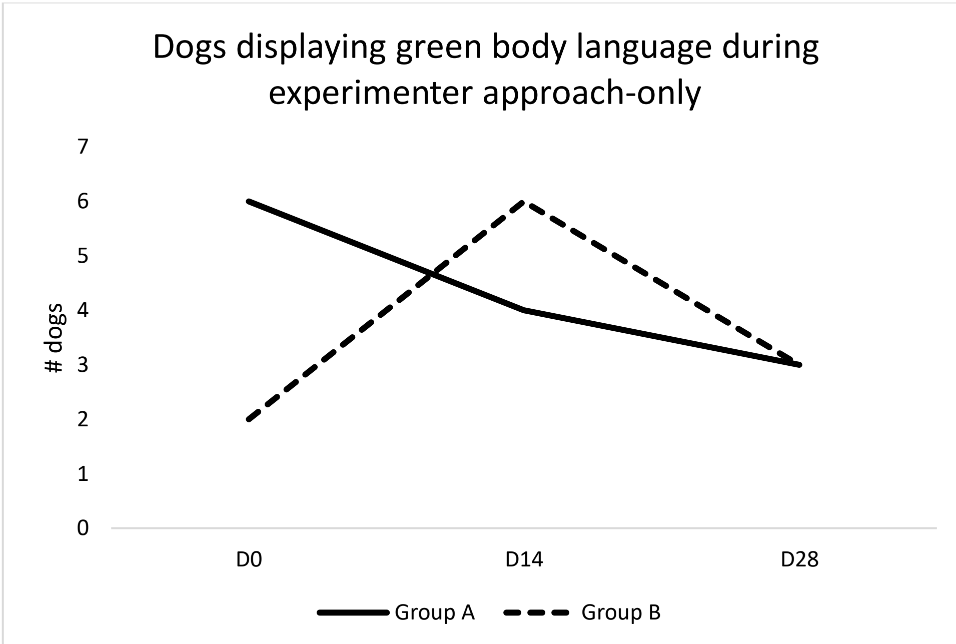


Figure 4.21. Descriptive representation of number of dogs displaying non-fearful (green) body language during the RYG approach only, in which dogs were free to go to the outdoor portion of the pen at Facility 2. The proportion of green dogs did not significantly differ over time; however, the mean RYG score significantly decreased in the CI group (Days 0-28, p=0.041), and significantly increased in the TO group (Days 0-14, p=0.044) over time.

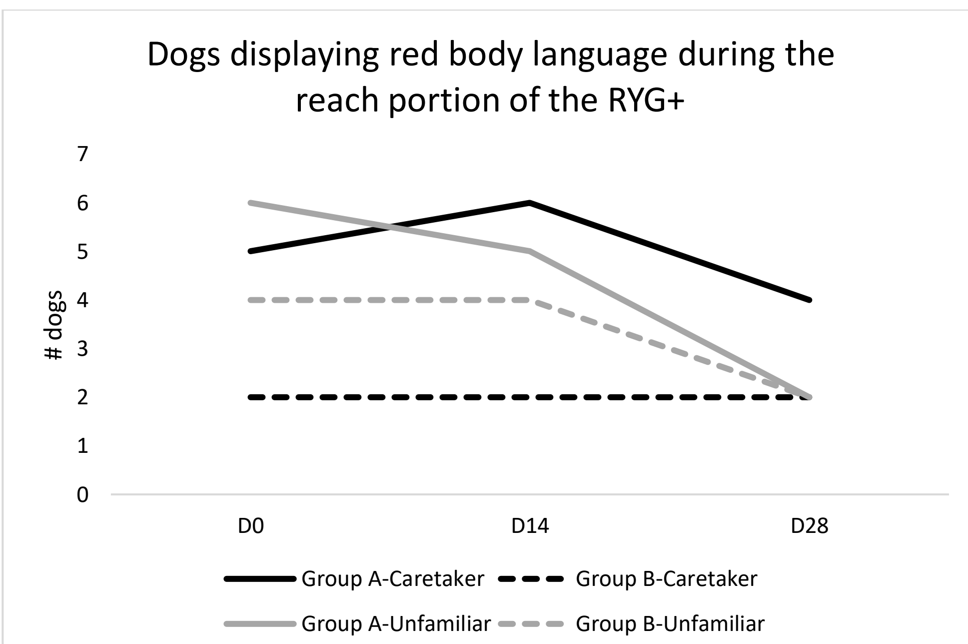


Figure 4.22. Descriptive representation of number of dogs displaying fearful (red) body language during the reach portion of the RYG+. A decrease in red body language indicates less fearful

body language over time. Mean RYG score significantly changed over time (X^2=13.578, *p*=0.001). The CI group's mean RYG responses to the caretaker's reach (X^2=4.305, *p*=0.037) and unfamiliar experimenter's (X^2=5.036, *p*=0.025) reach increased over time. The TO group's responses to the unfamiliar experimenter's reach increased as well (X^2=5.816, *p*=0.016). An increase in RYG score indicates less fearful body language over time.

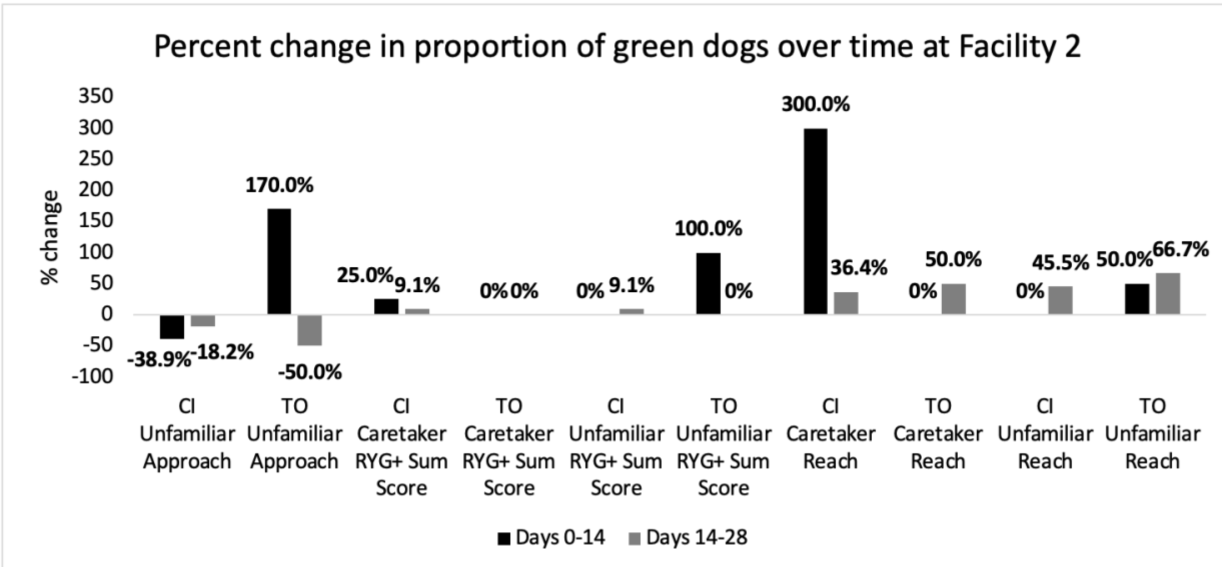


Figure 4.23. Percent change in proportion of dogs showing non-fearful (green) body language during human approach tests between Days 0 and 14, and Days 14 and 28 at Facility 2.

Daily caretaker interaction

The CI group did not show any significant changes in time spent displaying green or yellow body language. The TO group, however, increased in green body language (X^2=8.382, *p*=0.004) and decreased in yellow body language (X^2=10.186, *p*=0.001) over the course of the treatment (Figure 4.25).

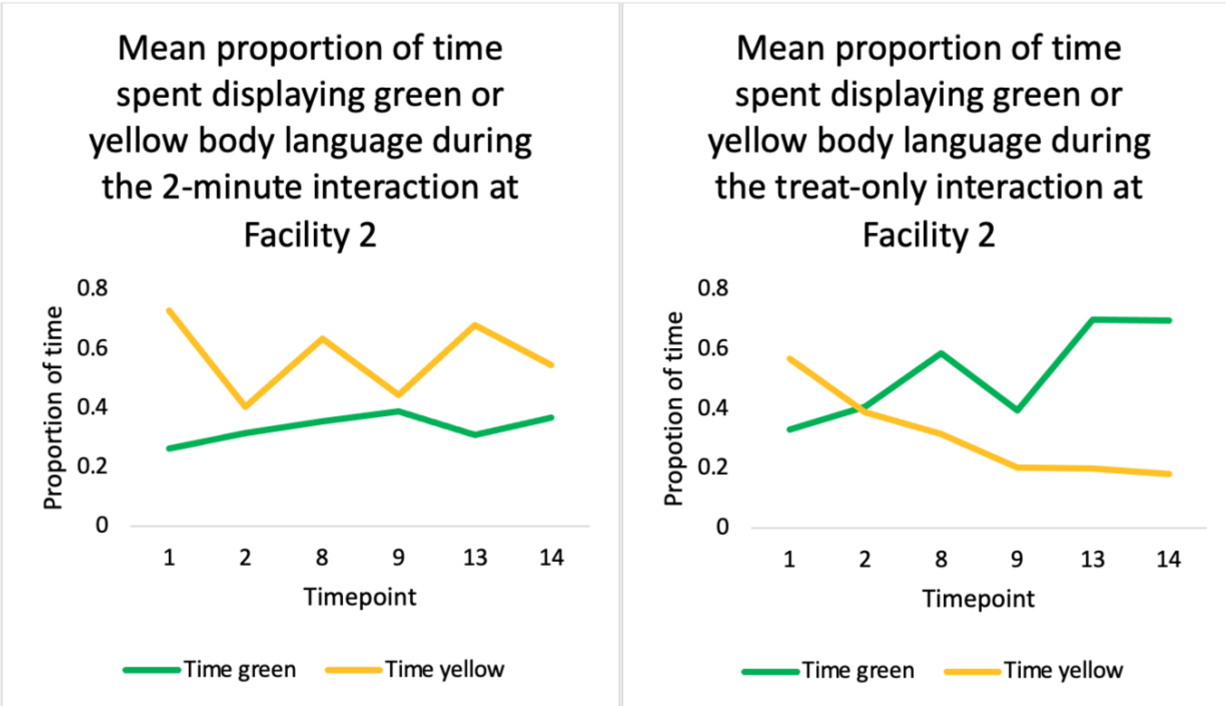


Figure 4.24. Proportion of time spent displaying non-fearful (green) and ambivalent (yellow) body language during the daily 2-minute and treat-only interactions. Time spent displaying green body language significantly increased (X^2=8.382, p=0.004) and time spent displaying yellow behavior significantly decreased (X^2=10.186, p=0.001) over time in the TO group.

4.2.3 Latency to Approach

Human approach tests

During the 1-minute reach portion of the RYG+, an interaction effect between treatment type, timepoint, and tester influenced latency to approach the tester (X^2=7.896, p=0.019) (Figure 4.26).

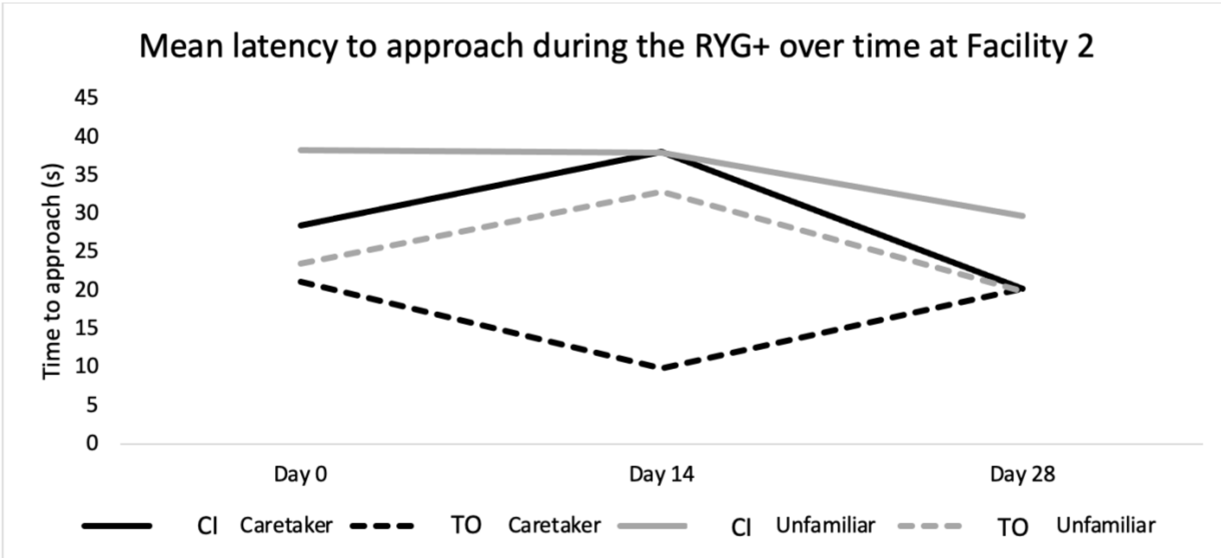


Figure 4.25. Mean latency to approach tester during the 1-minute reach portion of the RYG+ approach test over time at Facility 2. An interaction between timepoint, tester, and treatment type significantly affected latency to approach (X^2=7.444, p=0.006).

Daily caretaker interaction

In the CI group, latency to approach the caretaker during the interaction increased significantly over time (X^2=4.054, p=0.044), however only 6 of the 12 focal dogs approached the caretaker during treatment (Figure 4.27).

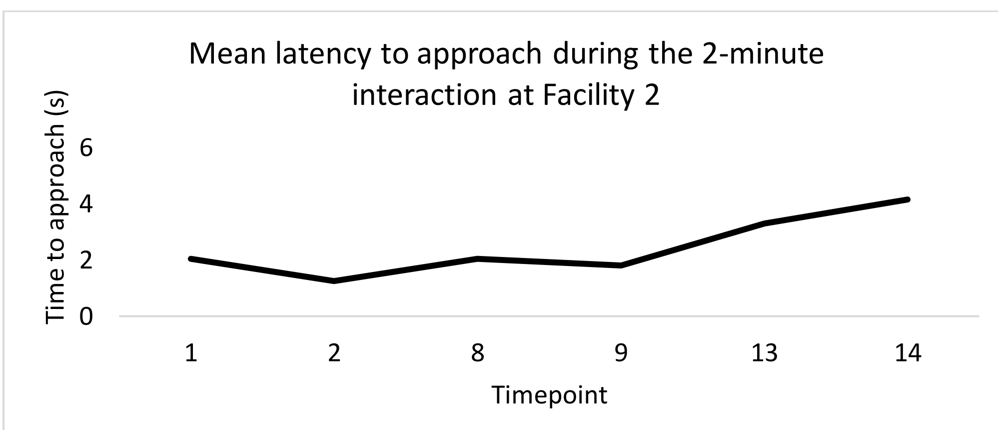


Figure 4.26. Latency to approach the caretaker during the daily 2-minute interaction. Six focal dogs approached the caretaker at each timepoint, increasing in latency to approach over time (X^2=4.054, p=0.044)

4.2.4 Interaction with Caretaker and Unfamiliar Experimenter

Human approach tests

During the 1-minute reach portion of the RYG+, proportion of time spent exploring the tester increased over time (X^2=8.266, p=0.016), with an interaction effect between treatment type and tester (X^2=5.819, p=0.016). Focal dogs spent significantly more time exploring the familiar caretaker than the unfamiliar experimenter (X^2=22.895, p<0.001). When examining treatment groups separately, CI dogs significantly increased in time spent exploring the caretaker only (X^2=4.249, p=0.039) and TO dogs did not differ significantly over time or according to tester (Figure 4.28).

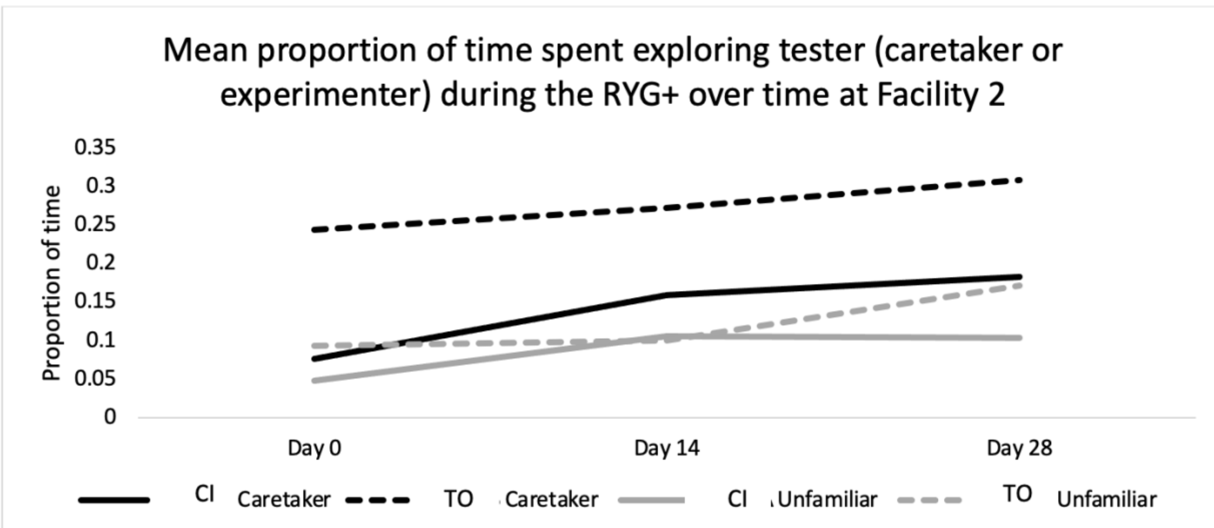


Figure 4.27. Mean proportion of time spent exploring the tester (i.e., familiar caretaker or unfamiliar experimenter) during the 1-minute reach portion of the RYG+ approach test over time at Facility 2. Proportion of time spent exploring the tester was significantly affected by an interaction between treatment type and tester (X^2=5.819, p=0.016), and increased over time (X^2=8.266, p=0.016).

Daily caretaker interaction

Time spent displaying affiliative behaviors (i.e., soliciting attention and seeking sustained contact with the caretaker) during 2-minute daily caretaker interaction (CI) increased over time (X^2=4.317, p=0.038; X^2=4.387, p=0.036, respectively) (Figure 4.29). Additionally, frequency of proximity to the caretaker increased significantly in the treat-only interaction (TO) (X^2=4.986, *p*=0.026).

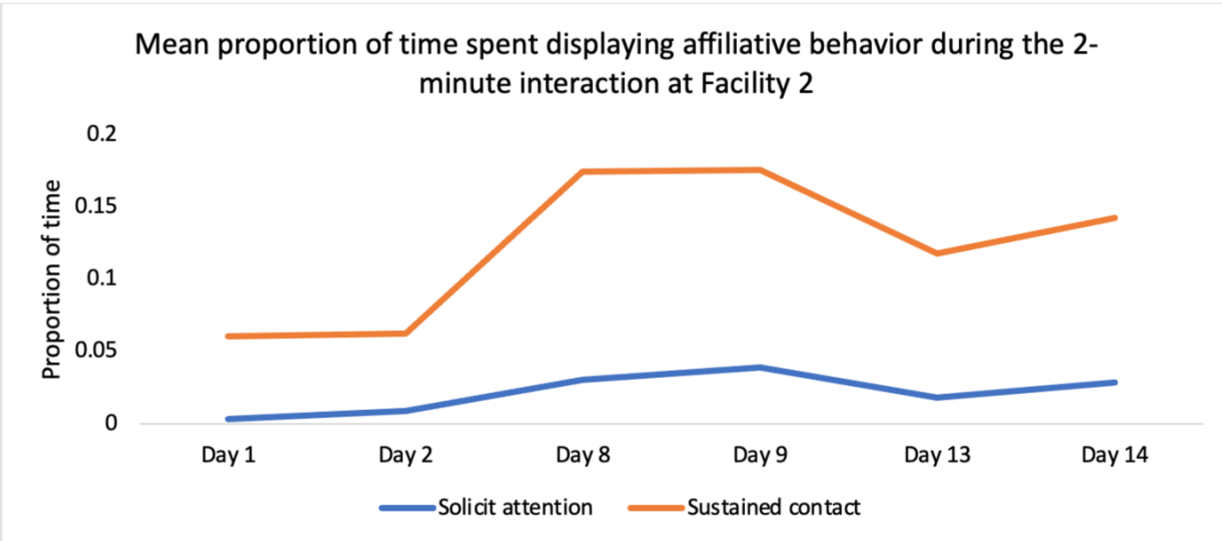


Figure 4.28. Proportion of time spent displaying affiliative behavior toward the caretaker during the 2-minute daily interaction. Time spent soliciting attention (X^2=4.317, *p*=0.038) and in sustained contact with the caretaker (X^2=4.387, *p*=0.036) increased over time, followed by a non-significant decrease toward baseline.

4.2.5 Preferred Location in Pen and Locomotion During the Daily Interaction

While proportion of time spent indoors, outdoors, or between did not change significantly over time, focal dogs in both treatment groups spent more time inside at all timepoints (Figure 4.30).

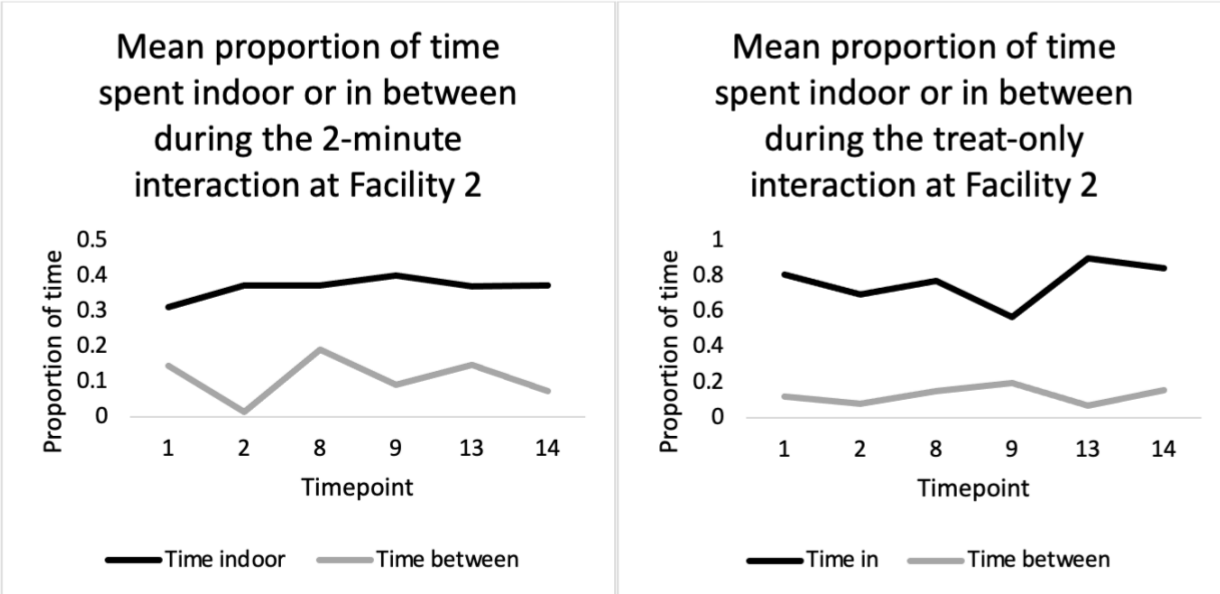
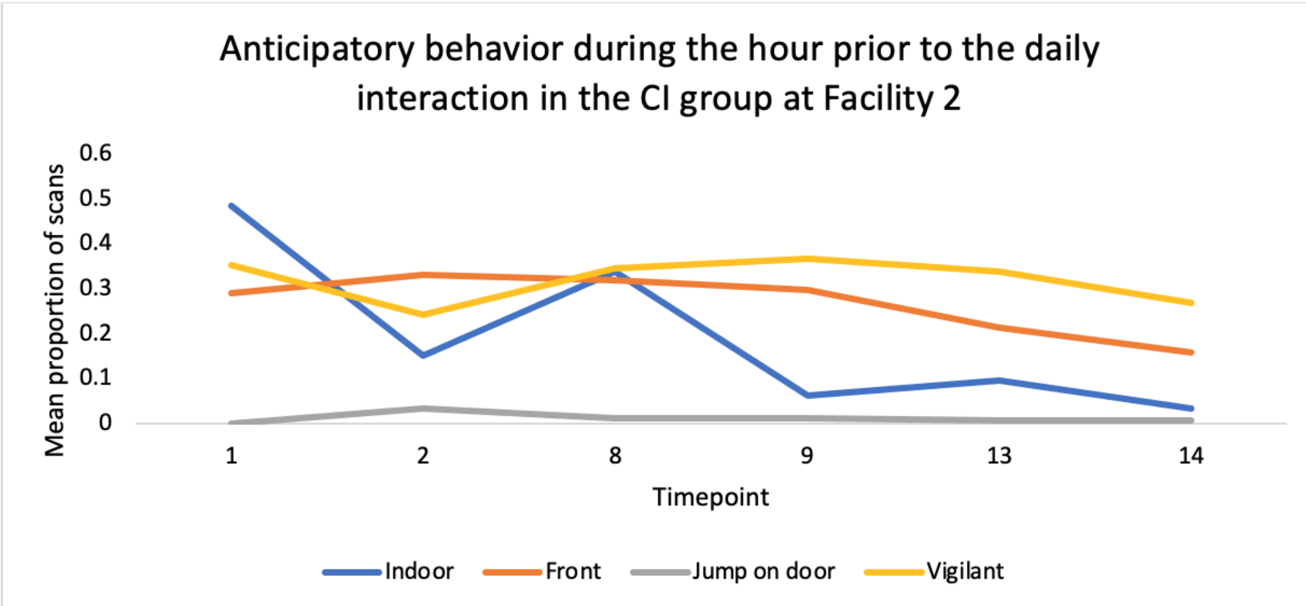


Figure 4.29. Proportion of time spent in the indoor portion of the pen and in between the indoor and outdoor portion of the pen during the daily 2-minute and treat-only interactions. Neither changed significantly over time.

4.2.6 Behavioral Time Budget Preceding the Daily Caretaker Interaction

Again, differences in behavior surrounding the interaction between hours (e.g., the hour before the interaction and the hour after) did not change over time. Certain anticipatory behaviors did change across timepoints, however. Time spent indoors, in the front portion of the pen, vigilant, and jumping on the pen door all decreased significantly over time in the hour prior to the daily interaction (X^2=101.797, p=0.000; X^2=20.555, p=0.001; X^2=131.447, p=0.000; X^2=20.755, p=0.001 respectively). Treatment type did not have an effect on any of these behaviors (Figure 4.31).



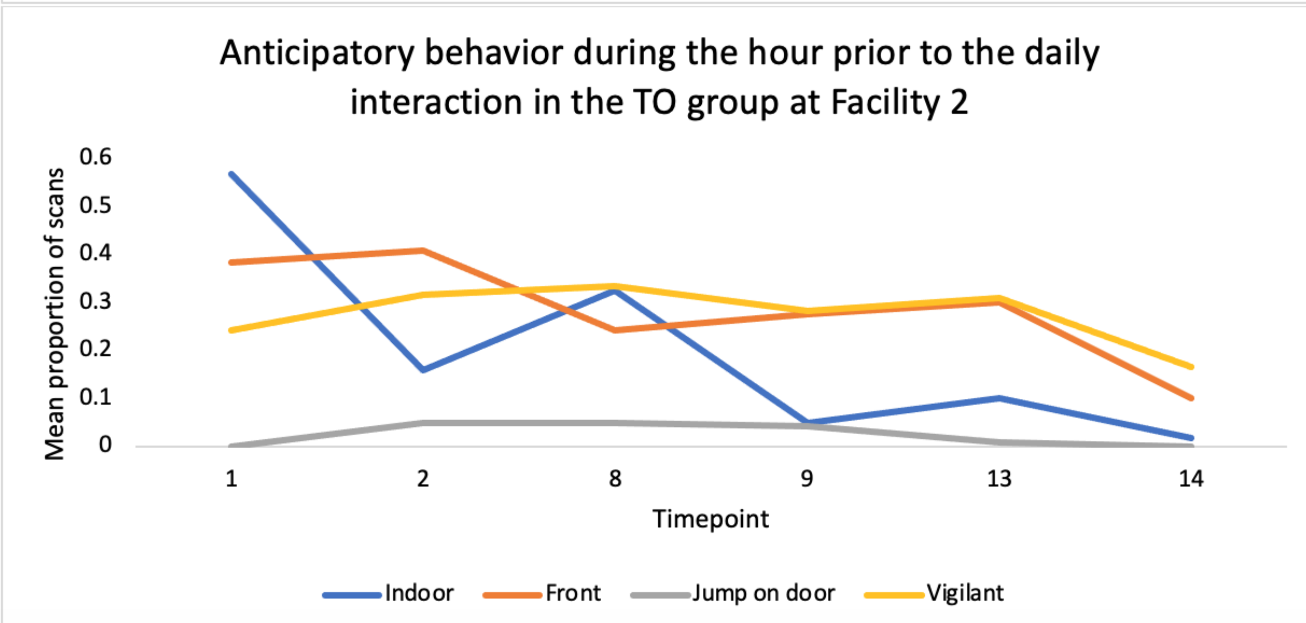


Figure 4.30. Mean proportion of scans observed in which focal dogs were performing various anticipatory behaviors in the hour prior to the daily interaction at Facility 2. (Indoor: X^2=101.797, p<0.001; Front: X^2=20.555, p=0.001; Jump on door: X^2=131.447, p<0.001; Vigilant: X^2=20.755, p=0.001).

CHAPTER 5. DISCUSSION

The objective of the current study was to determine whether a positive, daily caretaker interaction impacted the welfare of adult dogs residing in commercial breeding (CB) kennels. Several changes in metrics of welfare were observed, with direction of change varying according to facility and treatment group. The first hypothesis, that physical metrics of welfare would change after two weeks of a daily caretaker interaction, was not met, as physical health problems at both facilities were unremarkable to begin with and did not change significantly throughout the study. The second hypothesis predicting a change in physiological metrics after two weeks of the interaction, was facility dependent. Fecal secretory immunoglobulin A (sIgA) decreased, and hair cortisol concentration (HCC) increased over time in Facility 1. In Facility 2, HCC decreased with no significant change in sIgA. The third hypothesis, that behavioral metrics of welfare would change after two weeks of a daily caretaker interaction, was largely met in both facilities, although direction of change depended on metric and facility.

5.1 Facility 1

5.1.1 Physiological Metrics

Fecal sIgA can be used as a measure of stress, offering insight into the valence of that stress (i.e., distress or eustress) (Campos-Rodriguez et al., 2013; Staley et al., 2018). A single measure of fecal sIgA can represent acute stress within a 24-hour period, while multiple measures over time can represent chronic stress (Campos-Rodriguez et al., 2013; Staley et al., 2018). Generally, acute stress is said to upregulate the immune response, while chronic stress downregulates it (Campos-Rodriguez et al., 2013; Dhabhar, 2009); however, there are conflicting results in the literature regarding the direction of change in sIgA in response to stress. In the current study, fecal sIgA was collected at three timepoints: baseline (Day 0), immediately post-treatment, (Day 14) and two weeks after the treatment had ended (Day 28). The 2-minute caretaker interaction group at Facility 1 showed a significant decrease in fecal sIgA during the treatment and a subsequent, non-significant, increase in the two weeks after the treatment. The treat-only group decreased in fecal sIgA over time, continuing through two weeks after the treatment ended, indicating that the effects of the treatment on sIgA persisted after the treatment period for just the treat-only group. Alternatively, the increase seen in the 2-minute interaction group after the end of treatment could be an effect of frustration from termination of the treatment. If the treatment was a positive experience, the dogs may have experienced a slight increase in distress when they no longer received the daily interaction. However, if the treatment was perceived negatively, the increase back in the direction of baseline could be an effect of recovery after the treatment ended, although sIgA levels still did not return to baseline. It is important to consider how sIgA levels could have been affected by the 24 hours prior to collection, and by the two weeks leading up to sample collection. One day prior to post-treatment sample collection (Day 13) and long-term sample collection (Day 27), experimenters were present at the facility for re-marking of focal dogs for identification. It is possible this "re-marking" event acted as an acute stressor. Decreases in salivary sIgA have been found in response to acute stressors in dogs (Kikkawa et al., 2003; Svobodoba et al., 2014); and in rats, intestinal sIgA decreased after six hours of restraint stress, not returning to baseline levels until five days later (Ponferrada et al., 2007). However, when assessing fecal sIgA in response to an acute stressor in dogs, Walker and colleagues (2014) found fecal sIgA to increase when shelter dogs were separated from a familiar conspecific. Behaviors exhibited in home pens by the focal dogs suggested the event caused some degree of distress (Walker et al., 2014). Considering these findings, it is unlikely that the acute stressor from the previous day would lead to a decrease in fecal sIgA.

There is evidence to support that the decrease in fecal sIgA found over time in Facility 1 could be in response to chronic stress. Low levels of salivary sIgA were associated with stress-related behaviors in dogs (Skandakumar et al., 1995), and fecal sIgA in shelter cats displaying anxious behavior decreased over time after entry to the shelter (Gourkow et al., 2014). However, intestinal sIgA has been found to increase after periods of one week to one month of chronic stress in rats (Reyna-Garfias et al., 2010; Cui et al., 2016). Interestingly, adrenalectomized mice experiencing restraint stress showed higher levels of intestinal sIgA compared to controls, and adrenalized mice that received high doses of glucocorticoids over four days showed lower levels of intestinal sIgA than those that received low glucocorticoid doses. This suggests that presence of glucocorticoids influences sIgA concentration in the intestine (Jarillo-Luna et al., 2007). If this holds true for dogs, it is possible that the increased cortisol found in Facility 1 dogs influenced the decrease in fecal sIgA.

While fecal sIgA can provide insight as to the valence of a stressor, hair cortisol concentration (HCC) demonstrates level of arousal. Only HCC in the 2-minute interaction group at Facility 1 increased, indicating increased arousal over time in this treatment group. It is possible that this increase in arousal was due to the variable interaction times at this facility. The caretaker who performed the daily interaction varied the start time by a range of two hours due to scheduling constraints. Inconsistency in caretaker interaction, regardless of valence, has been found to increase stress in livestock (Hemsworth et al., 1987), cats (Gourkow & Fraser, 2006), and be associated with owner-reported behavioral problems in dogs (Luna-Cortes, 2021). It is possible that this inconsistency only induced a greater level of arousal in dogs in the 2-minute interaction group because of the longer interaction time, while the shorter, less invasive nature of the treat-only interaction allowed dogs in that group to remain sufficiently under the threshold of arousal so as to avoid an observed increase in cortisol. However, without examining these findings concurrently with behavioral metrics, it is not possible to know whether this arousal is of positive or negative valence. It is also important to note that in this facility, the caretaker elected to keep focal dogs from the exercise yard during treatment in order to keep them in their assigned pens, which differed from their usual management. This departure from routine may have caused an increase in distress during the treatment period, which could have introduced a confound into the observed changes in physiological metrics of welfare. For those seeking to implement new caretaker interaction protocols, it is important to consider the manner in which these protocols are introduced, taking special care not to disrupt regular management routines, thus potentially introducing unintended distress.

5.1.2 Behavioral Metrics

In this facility, the only change in behavioral response to approach occurred during the "reach", the third step of the human approach test, which was the most invasive portion of the test. For both treatment groups combined, mean RYG scores increased, meaning dogs were less fearful. As RYG scores in response to human approach outside of the pen did not change, it is possible that dogs in this facility were accustomed to approach by both familiar and unfamiliar people outside of their home pens. However, the action of a human reaching into their pen was novel. The increase in RYG score (i.e., red, yellow, or green body language) during this step of the approach test in the treat-only group suggests that dogs formed positive associations with human approach during the treatment. However, this increase in mean RYG scores was not observed in the 2-minute interaction group when treatment groups were examined separately in post-hoc testing. As the caretaker reached into the pen as part of the daily interaction, it is possible that the dogs in this group that showed green (non-fearful) body language during the reach step were the dogs that were already green prior to the treatment. If so, this would suggest that while the interaction may have been beneficial to these dogs, it did not decrease fear in those that were less gregarious or clearly fearful. Fearful dogs in the 2-minute interaction group tended not to elect to interact with the caretaker throughout treatment, thus having little ability to form a new, positive association with human approach. Similar results were found when Conley and colleagues (2014) tested the effects of a daily, 2-minute interaction in the shelter environment on salivary cortisol, behavioral metrics, and success on a behavioral assessment (Conley et al., 2014). While the authors found that treatment influenced certain behaviors in response to approach, it did not influence cortisol or the behavioral assessment. Instead, they suggested that while individual behaviors may have improved, they were not of substantial enough magnitude to change the outcome of the tests (Conley et al., 2014). Additionally, in the treat-only interaction, dogs who tended to be fearful in response to human approach had the opportunity to interact with their caretaker in a low-risk manner (i.e., with the pen door closed), while forming the association between human approach and the delivery of treats. This positive association appeared to generalize to an unfamiliar person, as similar results were found in response to unfamiliar experimenter approach. This generalization between familiar and unfamiliar people was also found in the above-mentioned study (Conley et al., 2014). Thus, it appears that the 2-minute interaction may not have been beneficial to fearful dogs, while the treat-only interaction may have offered a less threatening option for these dogs. Similarly, McGowan and colleagues (2018) found salivary cortisol to increase (albeit non-significantly) after a 15-minute interaction in dogs that chose not to engage with an unfamiliar experimenter (McGowan et al., 2018). For non-fearful dogs, however, the 2-minute interaction was likely beneficial.

Several changes in behavior during the 1-minute reach portion of the human approach test suggested an improvement in affective state while a human was present inside the pen. However, due to loss of baseline video recordings, caution must be exercised when interpreting these findings. Behaviors indicative of stress, such as lip licking and creating distance from the tester (Bauer et al., 2017; Beerda et al., 1999; Firnkes et al., 2017), decreased after treatment ended (between Days

14 and 28) in the 2-minute interaction group, suggesting a decrease in fear responses to human presence in the pen. Similarly, in the treat-only group, orientation toward the tester and vigilance (i.e., watching the tester) increased, suggesting another improvement in affective state occurred during the approach test (McGreevy et al., 2012). Dogs have been found to gaze at familiar humans in times of conflict or uncertainty, presumably to gain more information (Miklosi et al., 2003), and they avert gaze in fear (Bauer et al., 2017), suggesting that orientation toward the caretaker or experimenter is positive. However, it is unclear whether these changes were a continuation from baseline, or a rebound effect after the end of treatment. Based on results from RYG scores during the same step of the approach test, it is likely that this decrease in stress related-behaviors and increase in affiliative behaviors after the end of treatment are persistent positive effects, rather than a return to baseline.

It is interesting to note that in both treatment groups, changes indicative of a negative affective state were noted less frequently during unfamiliar experimenter approach than with the caretaker. For example, focal dogs in the 2-minute interaction group showed a higher frequency of lip licking during the caretaker “reach” step of the human approach test than during than the unfamiliar experimenter’s reach. It is possible that, while behavioral responses to the caretaker still improved over time, the dogs may have had learning history with their caretaker that influenced their responses, since caretakers need to reach for their dogs for husbandry, grooming, and medical procedures that might be perceived as positive, negative, or ambivalent by their dogs, depending on the specific nature of the interaction. The unfamiliar experimenter may therefore have been perceived as a more neutral stimulus (Chan & Harris, 2017). This suggestion is supported by the finding that latency to approach the tester decreased in response to the unfamiliar experimenter in both treatment groups, and only increased in response to the caretaker in the 2-minute interaction group. Therefore, it is possible that a more gradual approach and/or extended time of novel caretaker interventions may be required for dogs to show greater improvement in affective state with caretaker approach, while positive effects may translate to a neutral unfamiliar person more quickly.

In addition to changes in behavioral metrics during human approach tests, several improvements were seen during the daily caretaker interaction. Again, video recording of the first two days of treatment was lost, therefore the results should be interpreted with caution. Dogs were free to exit the indoor portion of their pens during the treatment, and the amount of time dogs elected to remain indoors increased over time in the 2-minute interaction group only. Additionally, green (non-fearful) body language increased while yellow (ambivalent) body language decreased in this group. Finally, time spent in proximity to the caretaker increased over time. Cumulatively, these findings suggest that there was an increase in positive affective state throughout the course of the treatment (Bauer et al., 2017). This conclusion is further supported by the increase in RYG scores during human approach tests at all three timepoints.

In the treat-only group, no significant changes in time spent indoors or green body language were seen. It is possible that an improvement in these metrics occurred prior to the beginning of video recording for this facility. Treat-only dogs did, however, increase proximity to the caretaker during the treatment and they reduced their frequency of exiting the indoor portion of their pens, again suggesting a positive effect of the treatment. The combined findings from the behavioral responses to human approach tests and the daily interaction observations suggests that the treatment was overall beneficial to dogs at Facility 1. While the 2-minute interaction group may not have experienced the same magnitude of improvement as the treat-only group, there was still a positive change.

Finally, in the hour prior to the interaction, anticipatory behavior tended to increase in both treatment groups during the second half of the treatment (Days 9 to 14). It is possible that once the focal dogs became accustomed to the new management routine, they began to anticipate the arrival of their caretaker. Anticipatory behavior is generally said to be indicative of positive emotional affect in expectation of a positive stimulus (Sprujit et al., 2001; Watters, 2014). However, at this facility, there were occasional inconsistencies in the time at which the daily interaction occurred. While anticipation generally precedes positive stimuli, anticipatory behaviors have been shown to decrease, along with stress-related behaviors, in response to increased predictability in husbandry routines (Gottlieb et al., 2013; Bassett & Buchanan-Smith, 2007). Therefore, while the interaction itself may have been largely positive, its unpredictability may have induced some distress, explaining the observed increase in HCC. Alternatively, the HCC might simply have captured positive stress (eustress) associated with anticipation.

5.1.3 Combined Examination of Metrics

The physiological and behavioral metrics collected at Facility 1 together suggest a change in dog welfare during the course of the two-week treatment that persisted in part through to collection of long-term post treatment metrics. The debate in the existing literature regarding interpretation of fecal sIgA makes determining its meaning difficult. However, in concert with the increase in HCC and improvement in behavioral metrics, it can be suggested that, for the dogs in this facility, the decrease in sIgA was a result of positive arousal (i.e., eustress), which has been documented in rats (Reyna-Garfias et al., 2010; Cui et al., 2016). The sharper decrease in sIgA in the 2-minute interaction group, along with the increase in HCC, suggests more arousal in this treatment group. Along with these findings, while both groups experienced improved behavioral metrics, the treat-only group showed more affiliative behavior in response to approach, which persisted after the treatment ended. These findings therefore suggest that for dogs that are unaccustomed to an interaction in which their caretaker spends time in their home pens, it may be more beneficial to begin with a less invasive interaction where their pen doors remain closed. After dogs become accustomed to this type of interaction, they may experience an increased benefit from a more involved interaction, which may contribute to a more positive overall welfare state.

An interaction two minutes in length was chosen as it was the shortest interaction in the literature documented to improve welfare in kenneled dogs (Conley et al., 2014). The choice to introduce a brief interaction was twofold: 1) the addition to routine management needed to be feasibly implemented in a CB kennel, where often one caretaker is responsible for daily husbandry tasks; and 2) because of the possibility that this population of dogs might not be accustomed to lengthy interactions with their caretakers due to time and personnel limitations, a short interaction was desired to avoid overwhelming the dogs. This serves to emphasize that even the shortest interaction in the literature, which was implemented in the shelter population, has the potential to cause unintended distress to dogs without due consideration of their previous experiences and learning histories, management of the interaction itself, and individual dog preference.

5.2 Facility 2

5.2.1 Physiological Metrics

In Facility 2, there was no significant change in fecal sIgA, however, as was observed at Facility 1, it decreased in both treatment groups. Hair cortisol concentration decreased significantly when both treatment groups were combined, but it did not significantly decrease for either treatment group alone. Unlike Facility 1, this represents a decrease in arousal over the course of the treatment (Grigg et al., 2017). It is possible that this new part of the daily management routine had a calming effect on the dogs. Alternatively, cortisol could have increased in the initial days of treatment at Facility 2, while the treatment was still novel, followed by a decrease in cortisol as the dogs' became habituated to their new daily routines, as the caretaker completed the interaction at a consistent time each day. This possibility is supported by behavioral findings discussed below. Finally, as HCC was not able to be measured immediately post-treatment, it is not known whether there was an increase or decrease during the treatment period.

5.2.2 Behavioral Metrics

RYG scores in the treat-only group increased (i.e., dogs became less fearful) in response to experimenter approach outside the pen (RYG approach-only) between baseline and post-treatment, suggesting a positive association having been formed to human approach outside the pen. Dogs and cats residing in shelters have been successfully conditioned to approach the front of their pens in response to human approach via positive reinforcement (e.g., Luescher & Medlock, 2009; Protopopova & Wynne, 2015; Grant & Warrior, 2019). Alternatively, the 2-minute interaction group at this facility exhibited a decrease in mean RYG score from baseline to two weeks after treatment ended, during the brief approach test in which dogs were free to exit to the outdoor portions of their pens. These dogs may have associated experimenter presence with the aversive event of being removed from their pen for collection of physiological metrics, as this occurred after the approach tests were completed at each timepoint. Livestock species have been documented to associate human handlers with aversive husbandry and display fear or stress-related behavior in response to approach (see Hemsworth, 2003 for a review). Because, during the 2-minute interaction, a positive stimulus (treat) was not introduced until after the pen door was opened, the 2-minute interaction group may not have formed the same positive association with human approach as the treat-only group. Thus, dogs in the 2-minute interaction group may have been more fearful in response to experimenter approach two weeks after the treatment ended (Day 28).

In response to the “reach” step of the human approach test, both treatment groups showed increased RYG scores over time, suggesting that dogs, on average, became less fearful. In the 2-minute interaction group, mean RYG score improved both in response to the familiar caretaker, and unfamiliar experimenter over time. Additionally, scores continued to improve after the treatment ended. In the treat-only group, RYG scores only improved in response to the unfamiliar experimenter, not the caretaker, at this step. Still, improvement persisted beyond the treatment. As was suggested for Facility 1, it is possible that dogs at Facility 2 may have associated their caretaker entering the indoor portion of their pens with aversive husbandry events, like veterinary care. The 2-minute interaction group may have been able to partially reverse that negative association via counterconditioning. Reduction in fear of humans has been accomplished via counterconditioning with positive human interaction in dairy cattle (Lensink et al., 2000; Lurzel et al., 2016) and silver fox cubs (Bakken, 1998). Over the course of treatment, the 2-minute interaction group experienced a positive event when the caretaker opened the pen door to give treats, while the treat-only group did not. Therefore, when the caretaker opened the pen door and reached into the pen for approach testing, dogs in the treat-only group may have continued to expect an aversive event, while the 2-minute interaction group had learned to expect something positive. Both groups may have shown improvement to the reach of an unfamiliar experimenter because of her neutral status and their mutual conditioning to expect treats when a human approached the pen. As animals of several species have demonstrated the ability to recognize individual humans and associate them with either positive or aversive events (Rushen et al., 1999; Koba & Tanida, 2001; Fell & Shutt, 1989), it is logical to conclude that focal dogs recognized and associated their caretaker but not the unfamiliar experimenter with certain experiences that may have been viewed as aversive or ambivalent.

The pattern of change in latency to approach the tester (caretaker or unfamiliar experimenter) during the 1-minute reach portion of the human approach test changed with an interaction effect between treatment type, tester, and timepoint. However, post-hoc testing on separate treatment groups yielded no significant results. Therefore, it is likely that the model contained too many fixed effects for this sample size. In terms of behaviors observed during the 1-minute reach portion of the human approach test, only dogs in the 2-minute interaction group significantly increased in time spent exploring the familiar caretaker. Again, it is likely that dogs in this group had been conditioned to expect treats upon the caretaker's entrance to the pen, thus eliciting exploration behavior. Additionally, while the increase in time spent exploring the caretaker tapered off after the treatment ended, it did not return to baseline. This demonstrates long-lasting, positive, residual effects on behavior in response to a familiar caretaker in the 2-minute interaction group. Similar results have been found with a positive training experience in horses (Sankey et al., 2010). Additionally, in cattle, a positive caretaker interaction reduced behavioral and physiological metrics of stress during a veterinary exam one week later (Waiblinger et al., 2004).

During the daily caretaker interaction, time spent indoors did not change over time; however, dogs consistently spent more time indoors than outdoors in both treatment groups. The dogs' preference to remain indoors for the treatment demonstrates that it was likely a positive experience (Kirkden & Pajor, 2006). In addition to electing to spend time indoors during the treatment, the treat-only group demonstrated an increase in green body language over time, supporting the suggestion that dogs in this group formed positive associations with caretaker approach outside of their pens.

In the 2-minute interaction group, latency to approach the caretaker during the daily interaction increased over time, which conflicts with the evidence in support of a positive association being formed with caretaker approach. However, it is important to note that the same six dogs in this treatment group approached the caretaker at each recorded timepoint, therefore the sample size for this metric is small. Additionally, the increase in RYG score and exploration of the caretaker during the "reach" step of the human approach test, along with the consistent choice to remain indoors during the interaction, suggests that the treatment was a positive experience overall. This is further supported by the overall increase in solicitation for attention and physical contact with the caretaker. It is possible that latency to approach increased over time in response to the conflict between reinforcement histories with the caretaker (Chan & Harris, 2017). Two weeks of a consistent, positive interaction in the indoor portion of the pen may not have been long enough to counteract the longer history of interaction in that location leading to a potentially aversive event, such as grooming or veterinary examinations. Future studies should examine a longer treatment period to determine whether this effect is temporary or if the increase in approach latency continues. Additionally, a caretaker interaction that takes place in the outdoor portion of the pen may yield different results, as this is often an area that is already associated with the positive event of being let out into the exercise yard.

While not as many positive changes occurred in the treat-only group, frequency of proximity to the caretaker increased over time. The lack of positive change in behavior in the treat-only group compared to the 2-minute interaction group may be due to the rapid nature of the treatment. With the caretaker simply dropping treats in the pen and moving away, the dogs did not have an opportunity to interact with him. It is possible that if the caretaker had remained in front of the pen for a longer period of time, similar changes in behavior would be seen.

Finally, anticipatory behavior (i.e., time spent inside in the front portion of the pen, vigilant, and jumping on the pen door) in the hour prior to the interaction decreased over time in both treatment groups combined. This aligns with the decrease in hair cortisol concentration noted above. It is possible that the interaction caused some initial arousal in the first 1-2 days, evidenced by an increase in certain anticipatory behaviors between the first and second day. That arousal decreased, however, when the interaction became routine. At this facility, the interaction happened at a very consistent time every day, which could have allowed the dogs to habituate more readily than if it had occurred at unpredictable times. Again, while anticipatory behavior is indicative of seeking an expected positive stimulus (Sprujit et al., 2001; Watters, 2014), it can suggest stress due to lack of predictability as well (Gottlieb et al., 2013; Bassett & Buchanan-Smith, 2007). Therefore, the decrease in anticipatory behavior observed at this facility may suggest a positive effect on welfare if the dogs were, in fact, habituating to the interaction.

5.2.3 Combined Examination of Metrics

Hair cortisol concentration was found to have a significant, direct effect on fecal sIgA. Considering that in Facility 1 there was an inverse relationship between the two metrics, and no such significant effect was found, this finding is likely an artefact of a small sample size. However, glucocorticoids have been found to influence intestinal sIgA in mice (Jarillo-Luna et al., 2007). More studies with a larger sample size are needed to determine whether this finding holds true.

In terms of behavioral metrics, the treat-only group did not demonstrate as much positive behavioral change over time as the 2-minute interaction group; however, there were no negative behavioral changes in this group. Additionally, the conflicting behavioral results in the 2-minute interaction group suggest potential ambivalence in dogs' affective responses to the caretaker. Anecdotally, the dogs that elected to interact with the caretaker at the beginning of the treatment tended to continue to do so throughout the study. Those that elected to remain outdoors while the caretaker was present typically remained outdoors at all time points. This, like the results from Facility 1, suggests that the treatment was beneficial to those that were not fearful of the caretaker at the onset of the interaction, and may have been at least somewhat aversive to fearful dogs. It is possible that some of the conflicting behavioral results are due to differences in the affective state of these two subgroups during the treatment. This suggests that, while the 2-minute daily interaction can be beneficial to the welfare of non-fearful dogs, a less invasive interaction (i.e., treat-only) should be done for more fearful dogs until they can become accustomed to this type of management.

5.3 Limitations

While important findings have certainly been revealed about the effect of caretaker interactions on the welfare of dogs in CB kennels, this study is not without limitations. Because only two facilities were studied, a larger sample size of kennels is required to confidently extrapolate the findings and conclusions to CB kennels in general. As management differs between facilities, the two facilities had to be analyzed separately to avoid the effect of facility masking effects of the treatment or timepoint. This highlights the importance of analyzing facilities on a case-by-case basis, as dogs at different facilities may respond to implementation of new interaction protocols differently due to factors, such as management, individual dog desires for human interaction, or learning histories with their caretakers that vary from facility to facility. Additionally, while utilizing each dog's baseline as its own control served to double the sample size, the study population within each facility was limited by inclusion criteria in place to protect the welfare of the focal animals (e.g., heavily pregnant bitches were excluded to avoid placing any undue stress on the dam or fetuses). This study should therefore be repeated at more CB kennels to determine whether patterns of changes in metrics of welfare persist in a larger population of dogs. Nonetheless, the consistency in the pattern of many of the findings across both kennels despite their many differences is promising in suggesting the likelihood of external validity if more kennels were studied.

In order to reduce the strain on the volunteer breeders' time, fecal sIgA was collected at pen level to avoid feeding each focal animal a fecal marker. While analyzing sIgA as a pen average is valid as treatment was applied to the pen, the sample size was decreased. Future studies should aim to administer fecal markers to all focal dogs to gain a clearer image of this metric. Additionally, there was no post-treatment (Day 14) measurement for HCC due to the fact that the dogs did not regrow sufficient hair for collection in two weeks' time. Interpretation of the change in HCC is limited as it may or may not be linear. As many changes in metrics of welfare began to return toward baseline by the collection of long-term post-treatment metrics (Day 28), it would be interesting to extend the treatment period by two weeks (for a total of one month) and assess persistence of treatment effects one month later (e.g., baseline: Day 0, post-treatment: Day 28, long-term: Day 56). This would allow for collection of HCC at the end of treatment, in addition to examination of whether effects would persist for a longer period of time if the treatment period were longer than two weeks. Additionally, fecal cortisol could be collected at two weeks after baseline as a more frequent long-term measure of stress.

Finally, a possible confound exists in dogs' responses to unfamiliar experimenters. While every attempt was made for experimenters conducting approach tests to remain novel to the dogs until the approach test, limitations on personnel required several of the same experimenters to return at each time point. In both facilities, focal dogs were re-marked for visual identification in the day prior to testing (i.e., Day -1, Day 13, and Day 27). This potentially stressful event may have influenced how dogs responded to experimenters the next day. Piglets have been found to exhibit a negative association with an aversive handler for up to five weeks after treatment (Brajon et al., 2015). Additionally, after approach tests, focal dogs were removed from their pens for collection of hair, blood (for a separate study), and a physical exam. It is possible that, even though each experimenter only conducted one approach test for each individual dog and did not handle that dog prior to testing, focal animals may have formed a negative association with experimenter presence and generalized that association to all unfamiliar experimenters. While several studies in the literature report animals to have recognized and associated individual people with previous events (Rushen et al., 1999; Koba & Tanida, 2001; Fell & Shutt, 1989), others have found animals to generalize unfamiliar people to both positive and aversive experiences with familiar people. Destrez and colleagues (2013) found that aversively treated lambs avoided both familiar and unfamiliar people (Destrez et al., 2013). Additionally, horses receiving a positive training treatment approached both familiar and unfamiliar people more quickly than controls (Sankey et al., 2010). Finally, no effect of familiarity was found when measuring dogs' approach to a human outside of their pen at a shelter (Conley et al., 2014). Fortunately, regardless of conflicting reports in the literature, positive change in behavioral response to approach still appeared to translate to unfamiliar experimenters in the present study. However, that change may have been of greater magnitude, or persisted for a longer period of time, if the potentially aversive collection of physical and physiological metrics did not happen concurrently with human approach tests. Further study should aim to conduct behavioral testing on a separate day prior to collection of metrics that requires removing dogs from their pens. Additionally, if personnel numbers allow, those that conduct behavioral testing should not be present during collection of physiological metrics.

Limitations notwithstanding, this pilot study provides evidence of improved physiological and behavioral metrics of welfare during and after a daily, positive caretaker interaction in two commercial breeding kennels. Just as importantly, the findings presented serve to emphasize the importance of the following: 1) consideration of the previous learning histories and management experiences of dogs prior to wide-spread implementation of caretaker interaction interventions; 2) the need for protocols that begin with brief interactions that gradually build in duration and intensity; 3) the importance of caretaker education to ensure consistency and quality of interactions with attention to low-stress handling and interactions with dogs; and 4) continuous assessment of the effects of implementing such protocols on the dogs in order to avoid unintended negative consequences. Recommendations made here can provide increased internal and external validity for further study. The methodology and findings presented will serve as an important foundation on which to build future protocols.

CHAPTER 6. CONCLUSION AND IMPLICATIONS

This study sought to determine whether a daily, positive, caretaker interaction would affect dog welfare in commercial breeding kennels. Results of this study show that overall, the daily caretaker interaction had a positive effect on welfare at both facilities. While physiological results differed according to facility, behavioral metrics appeared to be more consistent. The 2-minute interaction group tended to demonstrate a higher number of improved behavioral metrics in both facilities. However, the same group also showed some evidence of conflicting behavioral results (i.e., increase in solicitation for attention coupled by an increased latency to approach during the 2-minute interaction), while the behavioral changes observed in the treat-only group were exclusively positive. The 2-minute interaction seemed to have a positive effect on dogs who elected to spend time with the caretaker; yet may have caused distress to those who chose to avoid him. If so, there was benefit in those dogs being able to make a choice as not having that opportunity might have worsened their experience. Therefore, spending time conditioning dogs to a positive experience upon human approach to the pen prior to opening of the door may be more beneficial than an immediate interaction inside the home pen. Additionally, the manner in which the caretaker interacted with the dogs, while intending to be positive, may have been aversive (e.g., lightly clapping in the direction of the dogs to call them). If implementing an interaction protocol, caretakers should receive training on how to interact with dogs in this population. This is important to consider when recommendations or requirements are made for the quantity and quality of caretaker interaction in this population of dogs. Mandating forced interaction with animals, though well-intentioned, may have the unintended consequence of introducing distress to them instead of improving welfare. With careful consideration and scientific study of the implementation of these caretaker interaction protocols; management can be tailored to the specific population of dogs at each facility to introduce an interaction that is beneficial to dog welfare.

The next goal of the study was to investigate whether any changes in welfare persisted beyond the end of the 2-week treatment. Many welfare metrics continued in the same direction of change after the treatment ended. Still, other metrics did not follow this pattern and instead tapered off in the direction of baseline; but did not fully return to baseline values. This shows that effects of the 2-week long treatment persisted for at least two weeks after the treatment ended, which has important implications for implementation. If positive effects on welfare persist beyond the end of treatment, completing the interaction may not be necessary long-term, or on a daily basis. Furthermore, because effects of a long-term daily interaction in this population have not been scientifically assessed, mandating this permanent addition to husbandry routines could have unintended consequences. Like the 2-minute interaction discussed above, a long-term daily interaction could be overwhelming for dogs that are not accustomed to such management. These dogs may need time in between periods of daily interaction to adjust.

Finally, we examined whether changes in behavioral response to the caretaker after the treatment period extended to an unfamiliar person. The majority of behavioral changes in response to the approach of an unfamiliar person mirrored those of the familiar caretaker, meaning positive effects of the caretaker interaction may generalize to people unfamiliar to the dogs in this population. However, certain aspects of behavior during human approach tests suggested dogs were not as comfortable with an unfamiliar experimenter as their familiar caretaker. For example, in Facility 2, dogs consistently spent less time exploring the unfamiliar experimenter than the familiar caretaker during the 1-minute reach portion of the human approach test. Thus, while many aspects of response to unfamiliar experimenter approach improved after treatment, and continued above baseline after treatment ended, dogs in these facilities may still need socialization to unfamiliar people in order to avoid fearful responses when exposed to new people. Overall, the 2-week, daily caretaker interaction helped to decrease fear in response to approach of unfamiliar experimenters in these facilities. Therefore, when crafting plans for socialization of adult dogs in this population, time and personnel should be allocated appropriately given that a daily interaction with an unfamiliar person may not be necessary (or even feasible) to improve behavioral response to strangers.

The current study provides evidence that positive, daily caretaker interactions can improve several aspects of dog welfare. It also demonstrates that such improvements may persist beyond the actual interaction, and positive behavioral responses that result may translate to unfamiliar people. Limitations aside, this study serves as a foundation on which to build future caretaker interaction protocols, and an important call to scientifically assess related new protocols before implementation.

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