Emotions, mucosal immunity and respiratory disease in shelter cats

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MSc.
Abstract

In humans, the link between negative life events and susceptibility to the common cold is well documented. Further, interventions that enhance emotional wellbeing can stimulate local immunity and reduce incidence of respiratory infections. It is also clear that, in cats, a stressful event such as entering an animal shelter can increase susceptibility to upper respiratory disease (URD) and cause recrudescent disease in cats with subclinical infections (e.g. feline herpesvirus type 1). This study examined whether behavioural interventions could enhance emotional wellbeing, stimulate secretory immunoglobulin A (S-IgA) and reduce incidence of URD in shelter cats.

A three-dimensional model was constructed using behaviours observed in 34 cats for 5min/per h (days 1, 3 and 5), S-IgA and cortisol concentration quantified from faeces produced within 24 hours of each observation day. The model showed a significant contrast between two groups of behaviour indicative of high and low arousal of the emotional defence system and were labelled Anxiety and Contentment respectively. Anxiety behaviours such as hiding, flat postures, freeze, crawl, startle, and retreat from humans were significantly contrasted to Contentment behaviours: sitting quietly at the front of the cage while observing activities, sleeping or resting while lying on their side, eating, grooming, walking and friendly response to humans. The third dimension indicative of low arousal of the appetitive / reward system included persistent meowing, scanning, pacing, pushing and destroying items in the cage, redirected aggression and various escape behaviours (e.g., pawing at walls/ hanging on cage bars, pushing on door latch). Loge S-IgA was higher for Content cats (mean 7.1 ±0.5 loge µg/g) compared to Anxious (5.6 ±0.6 loge µg/g) and Frustrated cats (5.6 ±0.7 loge µg/g) (P <0.001) cats. Although several Frustration (pace and escape behaviours) and Contentment (walk, friendly response) behaviours correlated positively to cortisol, overall the Mean values were not significantly different for Frustrated (4.2 ±2.0 loge µg/g), Content (3.7 ±1.6 loge µg/g), and Anxious cats (3.0 ±1.0 loge µg/g) (Estimate 0.13, SD 0.31, Wald 1.67E-01, P =0.68). The multidimensional model labelled University of Queensland Indices of Cat Wellbeing (UQ-ICW) was subsequently used to rate emotional states in 250 cats upon admission to the shelter. Cats were assigned to one of 3 treatment type. Gentling, cognitive enrichment or positive human interaction was provided for 10 mins 4 times daily over a 10 day period to Anxious, Frustrated or Content cats respectively. Emotional and immune responses were measured for 10 days using UQ-ICW and quantification of S-IgA respectively. Ocular and nasal samples were obtained at intake and subsequently on days 4 and 10 to test for shedding of URD pathogens common in cats. In the Anxious group (n=139), gentled cats were more likely to be rated as Content (IRR: 1.52, CI: 1.27-1.81, P < 0.001) and had higher S-IgA (6.8 Loge µg/g) than Control cats (5.9µg/g) (P <0.0001).
Those responding positively to Gentling had higher Loge S-IgA (7.0 µg/g) than negative responders (6.1 µg/g) \((P = 0.001)\). Control cats were more likely to develop URD overtime compared to gentled cats (HR: 2.37, CI: 1.35-4.15, \(P < 0.0001\)).

In the Frustrated group \((n=15)\), cats provided with cognitive enrichment were more likely to be rated as Content (IRR: 1.93, CI: 1.01-3.68, \(P < 0.0001\)) and had higher Loge S-IgA than Control cats (6.7 µg/g versus 6.1 µg/g, \(P = 0.03\)). Those responding positively to treatment had higher S-IgA than negative responders (6.8 µg/g versus 6.0 µg/g, \(P = 0.003\)). Treated cats were less likely to develop URD overtime (HR: 2.37, CI: 1.35-4.15, \(P < 0.0001\)).

For cats classified as Content \((n=96)\), the positive interaction treatment increased the likelihood of sustained contentment compared with the Control treatment (IRR: 1.93, CI: 1.01-3.68, \(P < 0.0001\)). Loge transformed S-IgA levels was significantly higher for treated than for Control cats (7.28 µg/g versus 6.74 µg/g, \(P < 0.0001\)). Control cats were more likely to experience onset of negative affect (Anxiety or Frustration) than treated cats (IRR: 0.61, CI: 0.42-0.88, \(P = 0.007\)) and were more likely to develop URD overtime (HR: 2.9, CI: 1.30-6.67, \(P = 0.01\)).

Overall, 28% of cats were shedding one or more infectious agent related to URD upon admission. Twenty-two percent were shedding *Mycoplasma felis* (*M.felis*) and fewer than 3% were shedding calicivirus (FCV), feline herpesvirus-1 (FHV-1) or *Bordetella bronchiseptica*. Overall, viral and bacterial shedding increased by 9% and 11% respectively over the three sampling times (days 1, 4 and 10) and was not significantly affected by source, sex, sterilization status or age \((P > 0.05)\). Over 40 days post admission, the cumulative probability of developing URD was 1.7 times greater for strays than owner-surrendered cats and 3.3 times greater for cats that were shedders compared to non-shedders at entry to the shelter \((P < 0.001)\). FHV-1 shedders were at greater risk of developing clinical signs than non-shedding cats \((P = 0.002)\). Median time to clinical URD for shedders of FHV-1 was 6 days, compared to 11 days for non-carriers. There were no differences in shedding rate upon admission between cats assigned to treatment and controls \((P > 0.05)\) and no difference overtime for Frustrated and Content subgroups \((P > 0.05)\). However, there was a significant increase in shedding overtime in non-gentled cats (22%, 36%, 61% on days 1, 4 and 10, respectively) but not for gentled cats (35%, 26%, 32% on days 1, 4 and 10, respectively) \((P = 0.006)\).

Our findings suggest that negative emotional states such as Anxiety and Frustration reduce local immunity and that behavioural interventions can induce Contentment enhance IgA-mediated immunity and reduce incidence of URD in shelter cats.
Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Publications during candidature

Nadine Gourkow N, LaVoy A, Dean GA, Phillips CJC. Identification of emotions in shelter cats from their behaviour, with investigations of immunological and physiological correlates. Applied Animal Behaviour Science (Accepted: with revisions)


List your publications arising from your candidature using the standard citation format for your discipline. Divide your publications into sections as appropriate in your discipline e.g. peer-reviewed papers,

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**Publications included in this thesis**


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Contributions by others to the thesis

The conception and design of this research project, as well as, analysis and interpretation of data were achieved through discussions and consultations with the advisory team and collaborators, namely Professor Clive Phillips, Professor Gregg Dean.

Statement of parts of the thesis submitted to qualify for the award of another degree

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List of Abbreviations used in the thesis

B. *bronchiseptica*: Bordetella *bronchiseptica*

Ct: Cycle threshold

*C. felis*: Chlamydomphila felis

FCV: Feline calicivirus

FHV-1: Feline herpesvirus 1

*M. felis*: Mycoplasma felis

PCA: Principal Component Analysis

PNI: Psychoneuroimmunology

UQ-ICW: University of Queensland Indices of Cat Wellbeing

URI: Upper respiratory infection

URD: Upper respiratory disease

S-IgA: Secretory Immunoglobulin A

S-cortisol: Secretory cortisol or cortisol metabolites

RT PCR: Real-time polymerase chain reaction
Chapter 1: Literature review

1.0 Introduction

Upper respiratory disease (URD) is pervasive in shelter cats worldwide, and scientists agree that emotional stress is a major contributor to recrudescent disease in cats with subclinical infections and increases susceptibility to respiratory infection [1-4]. In addition to the discomfort associated with the disease, it is the second leading cause of euthanasia in shelter cats [6]. When cats do receive medical care, they may be subjected to extended periods of confinement in isolation wards [7] with minimal human interaction. The common occurrence of URD outbreaks in animal shelters [8], together with the day to day management of sick cats, represents a significant financial burden for humane organizations [9]. In North America, several guidelines for the care of animals in shelters have been produced to promote the use of environmental and social enrichment that may aid in enhancing emotional well-being. For example, The Association of Shelter Veterinarians produced the “Standards and Guidelines for the Care of Animals in Shelters” [10]. This document includes a detailed section on social and environmental enrichment, advancing that successful enrichment programs should provide for the psychological wellbeing of animals and that enrichment should be of equal importance with other required components of animal care, such as nutrition and veterinary care. Many scientists [11-16] argue the case that it is our moral responsibility to provide opportunities for positive affective experiences to animals under our care. Moreover, much evidence now shows that emotions are linked to immunity and health, and positive emotions, such as pleasure, are essential for good health.

I have hypothesized that the range of behaviours observed in shelter cats may be indicators of underlying emotional states that may affect the immune system differently and that manipulating these emotions with behavioural interventions may lead to enhanced emotional wellbeing, mucosal immunity and a reduction in incidence of URD.

This literature review presents firstly, a brief historical account of the scientific inquiries that contributed to the view that emotions and immunity are an integrated system of defence; and secondly, theoretical frameworks for the non-invasive measure of emotions that may be useful in identifying the emotion-immunity-health link in shelter cats. Thirdly, this literature review presents enrichments believed to alter emotions, immunity or both.
Chapter 1

2.0 Psychoneuroimmunology: An historical perspective

Over the last century, converging data from various disciplines has led to the birth of psychoneuroimmunology – “the study of behaviourally associated immunological changes and immunologically associated behavioural changes that result from reciprocal interactions among the nervous, endocrine, and immune systems” [17 p.1]. According these authors, defining the pathways and mediators associated with behavioural regulation of immunity, and understanding how manipulation of these pathways and mediators influence health and pathology are of obvious clinical importance.

Although many of the experiments presented in this historical account were highly invasive and objectionable to many [18-21], including myself, it is also true that the knowledge gained has contributed to a paradigm shift in veterinary (and related) sciences with positive implications for the welfare of animals. For one, view of animals as devoid of emotions [22-24] is now considered outdated [25] as it disregards the evidence of a shared phylogeny validating the similar capacity for affect in non-human and human animals [26, 27]. In addition, emotional pain is now paralleled to physical pain [28] and is believed to include a wide range of emotions such as fear, anxiety, separation distress, loneliness (isolation stress), boredom, frustration and anger. A growing awareness of animal emotions and their link to health is also evident in the shelter medicine field. For example, shelter medicine researcher Brenda Griffin [29] advances that the emotional states compromising health must be identified and addressed.

The earliest known proposal (in the Western world) of the notion that mental states could influence health was by Hippocrates (460–370 BC). He believed that disease was the result of an imbalance of the four humours (blood, phlegm, black bile and yellow bile) which were influenced by environmental factors, mental states and living habits [30]. This is illustrated in his famous quote that “it is more important to know what sort of person has a disease than to know what sort of disease a person has” [31]. Galen (AD 131–200) further expanded the theory of the four humours with a typology of psychological factors that may affect health. For example, he proposed that melancholic (introverted) women were more susceptible to disease than sanguine (impulsive and pleasure seeking) women. Hippocrates, Galen, and the physicians who followed during the sixteenth century sustained the notion that health and mental states were linked. It was not until the time of René Descartes (1596 –1650) that the mind and the body came to be seen as separate entities [32]. Descartes proposed a view of the human body as a machine with the only link between the mind and the body being the pineal gland at the base of the brain [33]. According to Easton
[34], an historian of modern philosophy, the orthodoxy instituted by senior professors to safeguard Galen’s ideas of the human body and anatomy prevented the dissection of human cadavers. Descartes’ conception of the body as a machine was important in challenging this orthodoxy in universities, which eventually lost its hold, contributing to much progress in the medical sciences [35]. Nonetheless, Descartes’ view gave rise to the denial of the mind’s role (emotions) in human health, a notion that influenced the modern medical paradigm until recently. Descartes also promoted a view of animals as machines devoid of emotions. He proposed that the squeals of animals represented mere mechanical reactions to external stimuli without any sensation of pain. In his publication “Part V, Discours de la Méthode” (1637), Descartes argued that “If a machine were made with the outward appearance of some animal lacking reason, like a monkey, it would be indistinguishable from a real specimen of that animal found in nature. But if such a machine of a human being were made, it would be readily distinguishable from a real human being due to its inability to use language” [36]. According to veterinary ethicist, Michael W. Fox [37], this view of animals had an important influence on veterinary medicine (p.237). However, Darwin’s renowned publications on the theory of evolution [38] and on the expressions of emotions in animals and humans [39] stimulated much scientific interest in the shared evolution of emotions across species.

2.1 The brain and emotions

During the 20th century, it was proposed that the brain may be the control centre for emotions. Walter Cannon [40] refuted the notion held by his mentor William James [41] that emotions occurred as a result of the awareness of visceral sensations in the body, such as increased heart rate and muscle tension. Rather, he proposed that an external stimulus, such as exposing a cat to a dog, elicited hypothalamic activity followed by a reaction in the central nervous system (e.g. increased heart rate) resulting in changes in behaviours (the fight-flight response). Following Canon’s work, James Papez [42] observed the trajectory of the rabies virus in the brain of cats and hypothesized that the interconnections between the hippocampus, the cingulate gyrus, the hypothalamus, and the anterior thalamic nuclei constituted a neural pathway of emotions. Over the next few decades, in addition to dogs, rats and primates, cats became the preferred subjects for investigations of neurophysiology which aimed to uncover the neural circuitry of emotions. Walter Hess (1881-1973) pioneered the technique of electro stimulation of the brain which revealed the synergistic coordination of mechanisms controlled by the nervous system with cerebrospinal innervated activities in the defence mechanism associated with emotional behaviours. His work mapping the brain of cats was cumulated in an atlas [43] with many illustrations of the specific areas brain structures involved in sensorimotor activities and emotions particularly the hypothalamus and
thalamus. Holger Ursin was the first to compared behaviours in tamed and feral cats elicited by brain stimulation [44] to those elicited by “real life” threats such as approaching, touching or restraining cats in a net [45]. He concluded that behaviours elicited by brain stimulation were undistinguishable from those elicited by real life threats. Subsequently, many studies provided consistent descriptions of fear (defensive attack), anxiety (defensive retreat) and frustration (restless and stereotypic behaviours) elicited by a variety of methods including hypothalamic stimulation, ablation or lesions and environmental stimuli [46-53]. It was also found that stimulation of the mesencephalic tegmentum (in the brainstem) which induced pleasure in human subjects (psychiatric patients) [54] could be used as a “rewarding” reinforcer for food consumption in aphagic (refusal to eat dry food) cats, and was proposed as a brain centre responsible for appetitive [55] and grooming behaviours [56, 57]. Interestingly, the infundibulum (uppermost part of the mesencephalic tegmentum) was identified as the area of the brain which controls purring [58]. It is now known that in many species including the cat, the nucleus accumbens and other limbic structures play a major role in reinforcement and motivational processes via the action of dopaminergic neurons which fire a burst of spikes when a reward is acquired and are inhibited when a reward is delayed, omitted or of lesser value than expected [59].

2.2 The psychoneuroimmune network

2.2.1 Stress and endocrine immunity

Evidence of a link between emotions and endocrine immunity had also been proposed by Hanz Selye [60], considered the father of the stress field, based on a series of experiments using 1500 female rats subjected to various stressors such as fasting, cold temperature, surgical injuries, restraint, and various drugs (e.g. morphine). All stressors, in particular the use of drugs and surgical injuries, were correlated with thymus involution and hypertrophy of the adrenals. The thymus is a major organ of the endocrine system where T-cells mature. To further investigate the possible role the adrenals may play in thymus involution, experiments were repeated using adrenalectomized animals. His findings showed that in the absence of adrenals none of the drugs or surgical injuries resulted in thymus involution. He concluded that a secretion of the adrenals must, therefore, be essential to the ability of the thymus to undergo sudden involution. Moreover, he found that after an initial enlargement, the adrenal decreases in size and returns to normal, even with repeated exposure to stressors. He concluded that the changes in the adrenals were not due simply to the presence of the toxic substance (in the drug studies), but were the morphological expression of an “alarm response” which enables the organism to meet critical situations efficiently. This was the
beginning of many subsequent experiments which led to the widespread acceptance of the concept of stress-induced immunosuppression. By the late 70’s, Selye [61] wrote “The Stress of Life”, a synthesis of 40 years of research on stress and immunity in which he defined stress as a non-specific response of the body to any demand for change, be it positive (he coined eustress) or negative, and advanced that long-term stress would result in disease. The interplay between environment, adrenal function and immunity is now well understood and has been the subject of much research in a wide range of species.

2.3 Behavioural conditioning of the immune system

Experimental work on the behavioural conditioning of the immune system [62, 63], based on Pavlov’s classical conditioning of behaviour [64], was well under way in France early in the 20th century. It was not until a landmark study by Ader and Cohen in 1975 [65] that classical conditioning of the immune system began to gain acceptance. An illness-induced taste aversion was conditioned in rats (including adrenalectomized animals) by pairing saccharin with cyclophosphamide, a temporary immunosuppressant. Subsequent presentation of the saccharin water prevented the production of antibodies in response to injected sheep erythrocytes. Immunologists were reluctant to accept the notion that cell-mediated immunity could be conditioned by altering behavioural responses [66]. However, these findings were replicated with similar results [67, 68] and built upon by evidence that humoral immunity could also be behaviourally conditioned [69]. These data support the hypothesis that stress hormones are key regulators of immune response. However, similar to the findings by Ader & Cohen, other researchers found that stress-induced changes occurred in adrenalectomized or hypophysectomised animals [70-72]. In addition, stress-induced elevations in glucocorticoid levels could occur in the absence of changes in immune function and stress-induced immune alterations in the absence of a change in glucocorticoid levels [70], indicating that factors other than adrenal output may be mediating immunity.

The notion that a wide range of emotions (positive and negative) may play an important role in immunoregulation and the pathogenesis of physical disease had been proposed by Solomon [73] based on the observations that symptoms of rheumatoid arthritis were exacerbated by depression. Solomon and colleagues [74] also proposed that abnormal immunologic factors may play a role in the pathogenesis of mental illness based on increased susceptibility of autoimmune disease in psychiatric patients. Postulation of a bidirectional network between emotional factors and immunity
prompted a flurry of research activity to uncover the mechanism by which various mental states may affect immunity and vice versa.

2.4 Communication network: The neuropeptides

Neuronal signalling molecules, now called neuropeptides (NP), are known to be the mechanism by which the brain and immune cells communicate [75]. Opioid peptides and their receptors were the first to be investigated [76]. Speculations that the body may contain receptors that bind to opium were based on observations of its potent influence on the body (e.g., control of pain) and the mind (euphoria) [77]. In the 70’s, opiate receptors were found on brain cells [76] and subsequently were also discovered on immune cells (e.g. T lymphocytes) in species ranging from primitive fish to primates [78-80]. The brain itself produces opiates (e.g., endorphins) which, similarly to the drugs morphine or opium, play an important role in the modulation of pain [81], behaviour [82] and emotions [83], but which can also trigger natural killer cells and support the immune system against diseases including cancer [84]. Converging data on behaviourally-associated immunological changes and immunologically-associated behavioural changes, and their communication system (the neuropeptides), led to the birth of psychoneuroimmunology [17] and psychoneuropharmacology [78]. In turn, this has led to new areas of medicine and pharmacology. Mind/body medicine (MBM) includes a wide range of therapeutic modalities grounded in the notion that the body can heal itself if a person is able to experience positive affect and remain stress free. MBM is believed to fill a major gap in contemporary health care which still denies the link between emotions and health [85]. In 2007, psychoneuropharmacologists Candace Pert, Michael Ruff and Michael Laznicka founded RAPIDPharmaceuticals (Receptor Active Peptides into Drugs) (www.rapidpharma.com) in the pursuit of developing peptide-based therapies (with neuropeptide analogues) for diseases such as dementia, psoriasis, AIDS, anorexia nervosa, libido and mood disorders. There are now at least 100 (mood altering) neuropeptides (www.neuropeptides.nl) identified in the mammalian brain [86, 87] known to exert considerable and reciprocal influences across all organs of the body including the immune system. Oxytocin, for example, is known to affect pleasure and bonding behaviour in many species including humans [88], rats [89] and dogs [90]. Oxytocin receptors in the thymus play a primary role in the maturation and differentiation of T-lymphocytes [91, 92]. Naturally or artificially elevated levels of oxytocin have been associated with increased wound healing in humans and mice [93, 94]. The neuropeptide glutamate has been implicated in anxiety and in the activation or suppression of various T-cell functions [95]. Neurokinin A, which affects drinking behaviour in rats [96], is also a chemo attractor for T-cells,
affecting their migration into infected tissues [97]. In cats, neurokinin A receptors were found in the amygdala (in all the nuclei except in the medial part of the central nucleus and the medial nucleus) [98], in the periaqueductal gray and in the brain stem [99], which are all associated with emotional behaviour [100]. Recently, it has been proposed [101] that, in addition to acting as neurotransmitters (released by one neuron and rapidly acting on an adjacent neuron), neuropeptides may act as hormones (released in the bloodstream and traveling to a distant site to produce long-term effects over a large area of the cell or over many cells). Thus, the author proposed that neuropeptides may be considered brain hormones [101]. For example, dopamine, sometimes labelled as the brain’s happy drug, is involved in motivation and reward/pleasure seeking behaviour in animals [102, 103]; and related to success and achievement in humans [104]. Deregulation of dopamine plays an important role in diseases such schizophrenia, Huntington’s disease, Parkinson’s disease, attention deficit disorder and autism [105].

As per the definition of psychoneuroimmunology, cells of the immune system also produce signalling molecules used in intracellular communication, which in turn influence behaviour. These types of behaviours (referred to as sickness behaviours) are believed to have evolutionary values that may facilitate survival of animals during disease [106], particularly infections [107]. According to Chang and colleagues, cytokines produced by leukocytes during infection and chronic inflammatory diseases serve as messengers that facilitate communication between cells of the mucosal immune system and the brain where they induce sickness behaviours. Prolonged activation of the immune system, such as during chronic infectious diseases, can cause symptoms of depression. Anorexia (not eating), which is commonly observed in sick animals, is believed to be an adaptive strategy for fighting acute infectious diseases motivated by the release of cytokines by immune cells and acting on the brain to induce inhibition of feeding behaviour [106, 108].

3.0 Non-invasive measures: Emotions and immunity

Many animal welfare scientists agree that the assessment of welfare should involve the determination of affect and measures of immune competence [109-111], and that non-invasive measures are to be preferred. Antibodies of the mucosal system that can be measured non-invasively by collecting bodily fluids may be useful biomarkers for positive and negative emotions [112, 113]. This section will review non-invasive measures of emotions in animals.
3.1 Mucosal immunity

Mucosal immunity is of particular importance for cats in animal shelters. It is the first line of defence against the URD pathogens [114] present at high concentration on shelter equipment, hands and clothing of caretakers [115] that can be inhaled or ingested by cats. The concept of localized immunity was first proposed by Besredka [116] at the turn of the 20th century on the basis of an experimental model for oral Shigella vaccination in rabbits. By the 1960’s, the secretory immunoglobulins (gamma-A in particular) were shown to be the principal source of viral antibodies in external secretions particularly in intestinal mucosa and salivary glands [117-119]. It was suggested that the secretory immunoglobulin A (S-IgA) was the major antibody class on all body surfaces and was part of a system separate from the humoral system for circulating antibodies. The mechanisms by which immunoglobulins (Igs) are transported to mucosal sites were then described by Tomasi, Brandtzaeg and their colleagues [120, 121]. S-IgA is now known to be the most prominent immunoglobulin of the mucosal immune system of cats [122-124]. It forms a glue-like substance which prevents penetration of bacteria, viruses and toxins across the epithelial wall. IgA deficiency is characterized by recurrent signs of URD in adult humans [125], children [126], and dogs [127]. Theoretically, a saturation of the IgA binding capacity occurs at higher concentrations [128] indicating the importance of stimulating secretion of IgA to promote protection against opportunistic URD pathogens. Similarly to other components of immunity, the mucosal immune system was also found to be modulated in part by emotions and stress. In 1977, the first large study to examine this link was conducted to examine the effect of release versus suppression of anger on the progression of breast cancer [129]. The authors found that release of anger could produce widespread metabolic alterations via the hypothalamus and autonomic nervous system resulting in increased production of IgA. In other species, acute and chronic stress also plays an important role in modulation of secretory S-IgA. In pigs, S-IgA rises in response to the acute stress of restraint [113] and decreases to below baseline levels in response to the chronic stress of confinement to single housing [130]. It increases when dogs from private homes enter an army kennel, then decreases after a few days [131]. In humans, mucosal immunity is enhanced in people with positive coping styles and in those with persistent feelings of happiness [132, 133]. Short term relaxation (10 min) [134] and listening to music [135] can increase the production of salivary IgA. The influence of emotional states on IgA secretion in cats has not been examined. However, feline IgA has been quantified in serum [136], in saliva [137], and in faeces [138, 139]. Emotional stress is a known risk factor for recrudescence of latent infections (e.g. Feline herpesvirus) [140] and immunosuppression in cats entering animal shelters [3]. Thus stimulating secretion of mucosal
antibodies particularly S-IgA by decreasing stress or inducing positive affect may be a critical aspect of infectious disease management in shelters.

3.2 Emotion Models

A recent survey by Izard [141] asked prominent emotion researchers from various scientific disciplines to define the word emotion. Many provided definitions that recognized the neural circuitry, phenomenal experience (feeling) and perceptual-cognitive processes of emotions. However, there was no consensus on a definition for the word. The author proposed that “The evidence showing that “emotion” has no generally accepted definition seems a clarion call for researchers who continue to use the term to provide their own operational definition, or at least specify what they mean by the term emotion” (p.370). Notwithstanding the anthropocentric views of animals which still cause much of the philosophical debate regarding the existence of animal emotion, (in my opinion) defining animal emotion according to known brain processes, and in the context of evolutionary function, may render the task of providing a definition simpler.

As suggested by Izard, I therefore clarify my “working” definition of emotions pertaining to this dissertation as: A biological process that can be triggered by external conditions meaningful to the animal’s sense of wellbeing which alters behavioural and immune responses such that the animal may attempt to meet survival, safety or comfort needs. I also propose that an adjunct word “dis-affect” may be needed to represent the deregulation of biological, emotional processes in animals due to the cruel and unusual conditions they still face in intensive farming, medical experimentation (among other) for which their evolutionary path has in no way prepared them (and to which they probably will never adapt).

Cross-species affective neuroscience [27, 142-145] and psychoneuroimmunology (section 2.2) certainly provide sufficient evidence to support this working definition of animal emotions without the bothersome task of having to defend their existence. Further, there is evidence that the limbic system evolved in the mammalian brain from basic mechanisms that gave them the ability to avoid harm or punishment (e.g. predators), and to seek valuable resources and rewards (e.g. feeding and mating opportunities) [27]. Since environmental conditions arouse emotional processes which alter behaviour, a trained observer may use behavioural clues within a given context to determine underlying emotional motivation. For example, purring behaviour in a cat being stroked by his owner may be interpreted as contentment, whereas purring following a serious car accident may be interpreted as distress [146]. Purring can occur in both of these situations with very different
meanings. Thus, the relationship between the context and the observed behaviour is important in determining the emotional experience of an animal.

3.3 The motivational model

The motivational framework approach [147] also called the affective valence model [148], proposes that emotions can be grouped according to their motivational systems. These are the appetitive (reward) system and the defence system [149]. The defence system is a fight or flight circuit initiated in response to danger cues. It mediates behavioural freezing, increased vigilance, and counter-threat displays in animal subjects. Appetitive motivation is characterized by varied forms of restless activity which serve to orient the animal toward a desired goal. Valence is the criterion used to describe the negative or positive propensities of the emotions aroused while intensity of emotional arousal is determined by the probability of nociception, or appetitive consummation.

3.4 Discrete Emotion Model

The affective valence model has been critiqued on both methodological and conceptual grounds in favour of a discrete emotion framework [150 p.411, 151, 152]. Some have argued that the affective valence model, in which emotions are psychometrically summed, may result in an erroneously similar interpretation of similarly valenced emotions (e.g., fear and anxiety) irrespective of their specific cognitive, physiological and health correlates [153]. Furthermore, they propose that a discrete emotion model can facilitate the understanding of the physiological, motivational, and cognitive pathways that link specific emotions to specific health outcomes. According to Izard [141], ”Future research on discrete emotions and their functions (when it can be appropriately contextualized and operationally defined), seems to hold unlimited promise for advancing psychological science” (p.370).

3.5 Appraisal Models

Cognitive theorists contend that thoughts and other mental activities play an essential role in the expression of emotions. Early cognitive theorists proposed that emotions, like judgments, are intentional: “an emotion always involves a personal evaluation of the significance” [154 p. 187]. Solomon proposed that (in humans) a change in judgment about a specific situation may produce a change in the corresponding emotions and associated physiology. This view is central to the current theories of appraisal, all of which assert that emotional responses are elicited in response to cognitive appraisal of a situation, and therefore account for variation among individuals in emotional reactions to the same event. Scherer [155] advances that appraisal is composed of three
sequential steps (called checks) which occur in a specific sequence: 1) sensory-motor, 2) schema-based and 3) deliberate. Each check includes relevance for novelty and relevance to goals, followed by an implication check for urgency, and a coping potential check. Although there are variations, Scherer’s model is, so far, the only one applied in animal research [16, 112]. Désiré and colleagues [156] found that the potential risks of a sudden or novel event can be evaluated by lambs, and that they are attentive to slight modifications of a situation according to their cognitive abilities.

3.6 Integrative Model

The motivational, appraisal and discrete approaches have been incorporated into a comprehensive dimensional model termed the Integrative Model of Emotions [5]. The model, illustrated in Fig.1, catalogues discrete emotions which oppose each other within the reward and defense motivational systems, with emotions in each dichotomized according to arousal level and valence. As illustrated in Fig. 1, high arousal of the defense system elicits fear and anxiety (quadrant Q4) whereas low arousal of the same motivational system elicits calm/relaxed state (quadrant Q2). Likewise, high arousal of the appetitive (reward) system elicits positively valenced emotions such as excited or happy (quadrant Q1) while low arousal elicits negatively valenced emotions such as sadness (quadrant Q3). Although the labeling of emotions in this model differs slightly from those proposed by affective neuroscience [142], the model provides a useful framework representative of current knowledge on the neural and biochemical activation of emotions. For example, acquisition of rewards activates dopaminergic neurons causing a release of dopamine which elicits pleasure [104, 157]. Conversely omission or delay of a reward causes a decrease in neuronal dopaminergic activity inhibiting learning [158] and eliciting apathy [159] or frustration [160].

Mendl and co-authors [5] propose that the Integrative Model can facilitate prediction of cognitive bias in animals according to their general experiences of success or failure in acquiring rewards and avoiding punishers within a given environment. “If an animal is in an environment in which it experiences frequent threatening events (emotional state is often in Q4), it may develop a negative mood state that mirrors this cumulative experience. If it is in a generally safe environment (or frequently successful at avoiding aversive events) it results in a positive mood state (Q2: relaxed /calm). Within the reward system, if the animal is in a plentiful environment and successful at acquiring fitness-enhancing rewards, it is likely to exhibit a mood state that is centred in Q1. On the other hand, a low-resource environment and failure to acquire rewards will lead to a predominantly Q3 mood “(p.2896). In humans, pathological anxiety or depression gives a tendency towards making negative judgements about ambiguous situations [161]. Similar findings have emerged in animals [162, 163].
4.0 Emotion-driven behaviour

4.1 Defense behaviours

Anxiety is a state elicited by uncertainty about potential risks, usually when faced with novel situations. It provokes hyper-vigilant behaviour (orienting attention towards the stimulus) and inhibition of normal activities such as eating, grooming, sleeping, and locomotion to facilitate appraisal [164]. Fear occurs when the stimulus is appraised as posing a real risk and is marked by flight, or defensive attack. In cats (when escape is not possible), fear is characterized by hissing, flattening of the ears, lowering of the body, drawing in of the head, piloerection, stiffening of the tail, and paw striking [165] with low intensity growl and hiss usually occurring just prior to attack [166]. Fear and anxiety (high arousal) are both elicited in response to threat, and the terms are often used interchangeably. In the context of confinement, the behavioural expressions of anxiety and fear (when no human is present) may be indistinguishable, because a fearful animal that cannot
escape may show similar hyper vigilant and freezing behaviour as an anxious animal. Although both represent arousal of the defence system, fear and anxiety are elicited by different perceptions of the threat. The difference may be due to the underlying mood, level of socialization (familiarity with humans), previous life experiences (exposure to novelty) or genetic disposition (boldness versus shyness). Both anxiety and fear represent a state of poor welfare with possible implications for health. Nonetheless, the distinction is important as they elicit different behavioural and physiological changes [164]. Most scientists agree that non-human animals experience fear; however, some argue that anxiety is part of the conscious (human) experience alone [167], because it depends on the ability to foresee the future or to imagine one’s own mortality [168]. In support of this view is the absence of anxiety, in the context of anticipated pain, in humans with prefrontal lobotomies which resembles the smaller, or virtually non-existent, cerebral lobes in non-human animals. However, Siegel reported that stimulation of the forebrain, including the cerebral cortex, is not essential to elicit defensive behaviour in humans and non-human animals [169]. In addition, receptors for benzodiazepine, known to play an important role in pathological anxiety in humans, have been found in many species [170], including domestic cats [171]. Benzodiazepines (Diazepam), effective in treating anxiety and panic attacks in humans [172], were shown to reduce heart rate [173] and defensive retreat behaviour in cats [174]. Lowering arousal of the emotional defence system is marked by normal sleep and rest patterns while lying on the side, and normal grooming and feeding behaviours [175 p.587]. Some behaviours associated with a calm/relaxed state are, however, context-dependent in terms of their emotional motivation. In cats, purring may indicate contentment, but can also be motivated by care soliciting (in kittens), sexual arousal (in females in oestrus) [176], and pain [177]. Normal sleep, particularly while lying on the side, may indicate a relaxed state [178]; whereas oversleeping may indicate apathy or depression [179]. Play has been linked to low arousal of the defence system [180, 181]. In cats, play is inhibited by overcrowding [165], the presence of perceived threats [182], and poor housing conditions [183]. Thus, the presence of play may also be indicative of low arousal of the defence system.

Another type of anxiety is commonly reported in companion animals which may not be related to the defence system. Cats are considered a social species in that they form social bonds, both with conspecifics [184] and with humans [185], which render them potentially sensitive to the loss of an attachment figure. In children, ambiguous situations can exacerbate anxiety with separation issues [186], and this may be relevant to cats. Indeed, many cats are diagnosed with separation anxiety [187]. It can be assumed that many cats entering an animal shelter had formed an attachment with their owner. Although not all may be predisposed to separation issues, those that
are may experience separation-related anxiety, in addition to anxiety induced by the novelty of the shelter conditions. However, brain imaging studies have shown that the limbic brain processes involved in human sadness [188] are also involved in separation distress in other animals [189]. In theory, sadness has evolved to facilitate adaptation to loss [190]. In humans, looking at a favourite person activates the dopaminergic system, with increases in immune response [191]. In cats, interaction with a familiar human has been shown to reduce arterial blood pressure [192], and may represent dopaminergic activity related to the neural reward system. Thus separation anxiety may be more akin to behaviours associated with the reward system.

4.2 Appetitive / Reward behaviours

The appetitive system modulates emotions which range from frustration (low arousal) to pleasure (high arousal). Frustration occurs under uncontrollable conditions, such as delay or omission of an expected reward (e.g. food) [193]; and thwarting of ethological needs, such as the inability to perform species-specific behaviours [194]. This emotional response may have evolutionary benefits, such as motivating an animal to initiate problem-solving activities to successfully achieve a desired goal [195]. For example, digging dirt to escape the confines of a yard is an effective strategy; however, the same behaviour performed by a dog confined to a cage does not facilitate escape and may result in stereotypic pacing or digging. When the behavioural repertoire of the animal does not successfully achieve a desired goal, it may be performed in a stereotypic (repetitive) pattern [196]. Pacing is common in confined carnivores due to their high motivation to roam [197]. Frustration is also expressed by engaging in behaviours seemingly unrelated to the goal [198]. For example, bar biting is common in tethered sows [199], and self-directed aggression has been observed in macaques [200]. The natural range of cats usually exceeds 200m$^2$ for house cats and 1.7km for feral cats [201]. In natural settings, cats engage in a wide range of behaviours (e.g. climb, perch, roam, hunt) and have a preference for eliminating and eating away from sleeping areas [202]. In domestic cats confined to the home, the sight of another cat outside can cause frustration and result in aggression redirected towards any available target [203].

There is considerable evidence that events not under the animal’s control are as important a source of stress as exposure to uncontrollable, unpredictable aversive events such as electrical foot shock [204], with similar physiological and immunological effects. Unpredictable husbandry routine, particularly with regard to feeding, increases cortical output in captive capuchin monkeys [205] and laboratory cats [206]. Uncontrollable foot-shock results in deficits in natural killer activity in mice, whereas controllable shock does not [207].
Conversely to frustration, pleasure occurs in response to reward; such as seeking and obtaining food, mating or other valued resources, or when the consequence of learning an activity (such as pressing a leaver) gives access to valued resources [25, 100]. Behaviours associated with pleasure in animals span from specific vocalizations (equated with human laughter) in rats [89] to play [181]. In humans, pleasure (and other positive emotions) have different health benefits than those incurred by the absence of negative emotions alone [208, 209] suggesting that positive affect may have characteristic biological correlates [210]. Similar notions are assumed with respect to animal well-being [112]. For example, pigs successfully trained to recognize and localize an individual acoustic summons for a reward showed a significantly higher concentration of immunoglobulins (IgG), increased in vitro T-cell proliferation to ConA, and faster wound healing than control pigs [211]. However, experimental evidence for the health benefits of sustained positive affect compared to the absence of emotional stress alone is sparse.

5.0 Enrichment

5.1 Enrichment and immunity

An extensive literature describes the negative effects of barren environments, social isolation, or overcrowding and poor handling on welfare whereas social or environmental enrichment and opportunity for instrumental learning is shown to improve welfare [212, 213]. Environmental enrichment (EE) aims to increase an animal’s opportunity to engage in natural behaviour (or possible approximations) and to prevent the development of abnormal behaviour [214, 215]. For example, artificial prey and olfactory enrichment has been used to stimulate hunting behaviour in wild felid species kept in zoos [216, 217], resulting in a reduction in stereotypic behaviour. Positive reinforcement training provided to primates was shown to increase cognitive ability and reduce aggression [218]. The behaviour of domestic cats living in homes and those living outdoors may be used for determining activities in which cats engage when given choices. Bernstein and colleagues [219] reported that in the home, cats may spend up to 5 hours observing activities through a window and tend to utilize most of the space available (particularly males). They tend to have various preferred sleeping areas chosen based on available warmth and prefer drinking [183] and eliminating [220] away from food sources. Cats are also known to enjoy play well into adulthood [165]. Other behaviours such as scent marking by scratching or rubbing on various substrates are also normal behaviours of domestic cats. Indeed, furniture scratching is a common behaviour for which owners seek help from behaviourists [221]. Cats that live outdoors can spend up to 34% of their time hunting [222] and engage in hunting even when provided with food. Thus, species-
specific enrichment for domestic cats should facilitate these behaviours [215, 223]. Interaction between pet cats and their human friends usually include pleasant physical contact such as touch, vocal interactions and spending time in close proximity [185]. However, in shelter cats, need for social interactions are likely to vary according to their personality, emotional state, age, and previous relationships to cats and people. For example, feral cats experience fear in their interaction with people due to poor socialization during kittenhood [224]. Thus, while environmental enrichment is likely to benefit all cats, social interaction (particularly those which involve touching a cat) may be a source of stress affecting the effectiveness of enrichment among individuals. In addition, individual needs of cats may change during their stay at a shelter. Cats may be coping well one week and be stressed the next [225]. In long term residents (7 years), aggression was found to increase overtime [226]. Notwithstanding possible differences in the effectiveness of enrichment due to temperament, previous life style and time spent at the shelter, it can be assumed that enrichment which effectively decreases stress and abnormal behaviour may also enhance immunity and health.

5.2 Environmental enrichment, immunity and health

In mice, EE that decreases anxiety behaviour (in the open arms of the elevated plus maze), increases activity (in the climbing staircase), and reduces freezing time in response to stressors was shown to also increase natural killer cell activity [227]. Elderly mice provided with toys and hiding areas for 8-16 weeks showed improved immunoregulation and decreased oxidative stress of immune cells compared to non-enriched mice (in barren housing) [228]. Although group housing can be stressful for rats (risk of overcrowding stress), females housed in small age-matched groups experienced enhanced cell-mediated immunity [229]. Not all studies have reported an immunological benefit in response to EE. One study that compared three EE conditions (a box, scaffolding or increased space) found no measurable immune changes associated with any of the EE provisions [230]. EE was also found to increase spatial working memory in mice compared to non-enriched mice with no difference in pro-inflammatory cytokines (following a peripheral injection of lipopolysaccharide). Moreover both groups experienced a similar degree of sickness behaviour [231]. In cattle, on the other hand, additional space and daily exercise were shown to reduce hock injuries and incidence of illness [232]. Similarly, dairy cows provided with loose housing and access to pasture have a lower incidence of lameness [233] and mastitis [234]. In shelter cats, stress has been associated with an increased risk of respiratory disease [9, 235]. Thus, the effectiveness of enrichment in enhancing wellbeing (emotional and immune health) is an important topic of investigation. For example, mice infected with mycobacterial agents and provided with a nest box and wooden chew block show
improved welfare, but no effect on immune response or prevalence of sickness [236]. Conversely, pigs presented with a challenging cognitive task had increased IgG, T-cell proliferation, and increased wound healing [211]. In humans, treatments such as watching a funny video increases dopamine, resulting in an increase in NK cell activity [191]. The effect of visual enrichment (television) was examined in shelter cats and shown to increase grooming and resting behaviours, and relaxed postures [237]; however, effects on immunity were not measured.

5.3 Social support, therapeutic touch and health

Social support - the perception that someone cares for you [238] can buffer the effect of stressors particularly in older people [239] and children [240] and can have a positive effect on health [241]. The notion that the presence of a familiar person may have similar effects on animals is supported by two areas of research. Firstly, separation anxiety is a common affliction of both dogs and cats [187] indicating that the presence of a familiar human to whom the animal is attached is comforting. Secondly, presence of a familiar human was shown to reduce the effect of a stressor. During an aversive condition such as receiving a foot shock, the presence of a familiar person has a positive effect on heart rate of dogs [242]. In most animal studies social support is referred to as positive human interaction. In shelter dogs, positive interactions with a familiar human can reduce heart rate and adrenal output and promotes calm behaviour [243, 244]. In cats, arterial blood pressure decreases when petted by a familiar person but does not change when petted by an unfamiliar person [192]. The physiological correlates of touch such as massages have a direct influence on immunity. In humans, massages can increase S-IgA [245] decrease cortisol, increase serotonin and dopamine levels [246]. Similar effects were found in animals. Gentle stroking decreases heart rate and increases oxytocin in rats [247]. Gentling stimulates S-IgA in lamb under artificial rearing conditions known to normally induce stress and immunosuppression [248]. The known health benefits of social support for humans and several other species suggests that treatment which involve positive social interactions may facilitate health welfare for cats in animal shelters as well.

6.0 Conclusion

This review represents a small portion of the vast literature related to the link between emotions and health. However, its focus on knowledge gained about the link between emotions and immunity based on animal models provides a strong foundation in support of the investigation of feline emotions in the shelter environment. Significant strides have been made towards improving the welfare of caged cats. However, there remains a gap in knowledge concerning the effect of enrichment strategies on emotions, immunity and health. In light of the known relationship in other species between affect, immunoregulation, and health, similar inquiries with cats may further
advance the fight against URD in animal shelters. There are challenges to overcome. For one, identifying the emotions of animals is methodologically challenging. Evidence supporting the appropriateness of non-invasive measures for the investigation of animal emotions and their physiological correlates is sparse. Moreover, in shelter cats, the medical history, the genetic and socialization backgrounds (which influence temperament), and previous lifestyle are often unknown. All of which may play an important role in emotional responses, immunoregulation and health. Despite these challenges, it is suggested that research which aims to decrease upper respiratory disease in cats may be most successful if it more thoroughly explores the emotion-health link.

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Chapter 1


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Chapter 1


Chapter 2: Multidimensional modelling of emotional states in shelter cats from their behavioural, immunological and stress responses.

Abstract

The purpose of this study was to construct a multidimensional model of emotional states that may be used for the assessment of emotional states of cats during their first week at an animal shelter. Thirty-four cats were observed for 5min/per h from admission to day 5. Physiological correlates of emotions secretory immunoglobulin A (S-IgA) and cortisol were quantified from faeces produced within 24 hours of observation days. Graphical display of the data obtained by a series of principal component analyses (PCA) revealed three emotional dimensions consistent with motivational constructs of Anxiety, Frustration and Contentment. Anxiety characterized by hiding, at the back of the cage, flat postures, freeze, crawl, startle and retreat from humans significantly contrasted behaviours of the Contentment dimension. These were sitting quietly at the front of the cage while observing activities, sleeping or resting while lying on their side, eating, grooming, walking, rubbing and friendly behaviour towards humans. Frustration was characterized by persistent meowing, scanning, pacing, pushing and destroying items in the cage, various escape behaviours and redirected aggression. Loge transformed S-IgA values were significantly greater ($P<0.001$) for Content cats (mean $7.1 \pm 0.5 \text{ loge } \mu g/g$) whereas they were not significantly different ($P>0.05$) for the Anxious ($5.6 \pm 0.6 \text{ loge } \mu g/g$) and Frustrated ($5.6 \pm 0.7 \text{ loge } \mu g/g$) cats. Overall, cortisol values were not significantly different for Frustrated ($4.2 \pm 2.0 \text{ loge } \mu g/g$), Content ($3.7 \pm 1.6 \text{ loge } \mu g/g$), and Anxious cats ($3.0 \pm 1.0 \text{ loge } \mu g/g$) (Estimate 0.13, SD 0.31, Wald 1.67E-01, $P=0.68$). However, cortisol was significantly greater ($P<0.002$) in cats that were engaged in pacing, and redirected aggression (Frustration) or walking and friendly response to humans (Contentment). These results suggest that behaviours and S-IgA may be suitable indicators of emotional states in shelter cats.

Key Words: Behaviour; Biplot analysis, Indices of wellbeing; Secretory cortisol; Secretory immunoglobulin A; Shelter cats.
1.0 Introduction

Animal health is commonly conceptualized as the absence of disease [1, 2] and emotional health commonly conceptualized in terms of levels of stress [3]. There is now a growing interest in expanding these concepts to include a broader range of parameters particularly emotions and immunity. Suggested concepts include emotional wellbeing, wellness, positive welfare and psychological health [4-8]. In common is the notion that emotions and health are linked and that positive emotions play an important role in maintaining health. Indeed, some veterinary scientists suggest that emotional states that may be compromising health must be assessed and addressed [9-11] and animal welfare scientists suggest that the assessment of welfare should involve the determination of both affect and immune parameters [12, 13]. The construction of behavioural indices to determine wellbeing (emotions and health) is a worthy effort, although it is not without conceptual and methodological challenges. An important first step in the construction of indices is the selection of relevant and measurable variables [14, 15].

Psychological stressors inherent to animal shelters and that may impact wellbeing include confinement, restraint and medical procedures, exposure to unfamiliar people and cats, barking dogs, unpredictable husbandry (particularly feeding schedule), and overcrowding. How a cat perceives these stressors may influence emotional, stress, and immune responses with possible implications for health. Upper respiratory disease is a significant problem for cats in animal shelters. In addition to the high concentration of respiratory infectious agents on shelter surfaces, hands, and clothing of caretakers [16] which puts much demand on the immunne system, the stressors encountered daily reduces their ability to fight pathogens that can be inhaled or ingested. As the mucosal immune system is the first line of defence against opportunistic pathogens [17], this aspect of immunity is extremely relevant to the health of shelter cats. However, similarly to other component of immunity, local immunity is affected by both positive and negative emotional states. According to motivational theory, emotions can be dichotomized according to arousal of two motivational systems. The defence system is a fight or flight circuit initiated in response to danger cues, whereas the appetitive (reward) system serves to orient the animal toward a desired goal [18, 19]. The arousal of the defence system in response to the presence of threat initiates emotional states commonly labelled anxiety and fear; whereas low arousal (in the absence of threat) elicits calm, content states [20]. Conversely, stressors such as the thwarted access to desired resources (by omission or delay) and inability to engage in behaviours the animal is motivated to perform can elicit frustration and aggression whereas acquisition of resources induces pleasure [21, 22]. Animal
shelter stressors (e.g., confinement and novelty) have the potential to elicit any of these emotions, depending on the animals’ cognitive appraisal of the stressors [23]. The identification of feline emotions has been the subject of much research since the influential work conducted by Hess [24] on the emotion-driven behavioural responses of cats to hypothalamic and thalamic stimulation. The notion that these structures are vital to both emotion and motivation has been widely investigated [25-27]. Further, some studies have shown that behaviours elicited by brain stimulations are qualitatively similar to those obtained by exposure to “real life” situations [28-30]. Thus, behaviours described in the many brain stimulation studies conducted on cats may be useful in qualifying emotional arousal of cats in response to stressors in the shelter environment. Two types of defence behaviour (attack and retreat) have been elicited by stimulation of different areas of the hypothalamus [31, 32] which corresponds to ethological constructs of anxiety and fear [33]. The authors emphasize that these responses are evoked by different perception of threats. Anxiety indicates uncertainty about the potential risk and thus elicits behaviours aimed at appraising risk and options available. Fear indicates that the animal has determined that the threats pose a real danger which elicits a flight / fight response.

The relationship between emotions and mucosal immunity has not been examined in cats. However, brain stimulation studies have that shown that fear (defensive attack), anxiety (defensive retreat) and restlessness have different effects on cell mediated immunity [34, 35]. Specifically, the authors reported an increase in lymphocyte proliferation in response to an injection of the toxin phytohemagglutinin in cats showing restless or defensive attack behaviour but not in cats expressing defensive retreat behaviour even though all experienced an increase in plasma cortisol. An explanation for the differentiated effect of emotions on immunity despite the elevated cortisol in all cats may have been various neuropeptides. These biochemical messengers bind to specific receptors in the brain (where they affect behaviour and emotions), the central nervous system (where they affect metabolism) and on immune cells (where they affect health) [36, 37]. Currently, there are at least 100 neuropeptides which singly, or with co-peptides, can exert significant effects on behaviour, emotions, metabolism, and immunity simultaneously [38]. For example, administration of exogenous neuropeptide bombesin into the rat brain causes grooming and satiety; and appears to modulate stress, fear, and anxiety [39]. This neuropeptide is also known to enhance IgA-mediated immunity in mice [40]. Positive emotions may similarly activate neuropeptides in cats that may enhance local immunity. S-IgA inhibits colonization and penetration of bacteria, viruses and toxins through the mucosal membrane [41-43] and is therefore essential for protecting cats against pathogens responsible of upper respiratory disease (URD) [44-47]. Conversely, IgA deficiency is characterized by recurrent signs of URD in adult humans [48, 49], children [50] and
dogs [51]. In cats, 80% of IgA-producing cells are found in the digestive tract [17, 42], as opposed to serum [52] and saliva [53]; and S-IgA is, therefore, prominent in faeces [54]. In other species, this antibody was found to be responsive to both acute and chronic stress. In pigs, S-IgA rises in response to the acute stress of restraint [55], but decreases in response to the chronic stress of confinement to single housing [56]. S-IgA increases in dogs upon entering an army kennel, but is inhibited after extended confinement [57]. Moreover, interventions such as gentling and massage, laughter, and relaxation were shown to increase IgA secretions in lambs and humans respectively [58,59-61] suggesting that manipulating emotions can enhance IgA mediated immunity.

Although the concept of emotional wellbeing is the focus of various veterinary books and guidelines for the care of shelter cats [8, 62], underlying emotions which alter behaviour and immunity remain unexplored in cats. Therefore, this study aimed to identify various emotional responses of cats during their first week at an animal shelter and to examine associated levels of faecal cortisol and S-IgA.

2.0 Method

This study was approved by the University of Queensland Animal Ethics Committee (CAWE/231/10).

2.1. Animals

Forty cats surrendered by their owners or entering as strays into an animal shelter (British Columbia Society for the Prevention of Cruelty to Animals, Vancouver, Canada) during April and May, 2010, were enrolled in the study. Cats were examined at intake by a Veterinary Health Technician. Vaccination was delayed until the end of the study (day 7); however, cats were dewormed (Strongid® T. Pfizer, Quebec, Canada).

2.2. Housing

All cats were housed individually in stainless steel cages (76 x 76 x 71 cm) furnished with litter boxes and non-absorbent cat litter (Veterinary Concepts, Wisconsin, USA). Stainless steel food and water bowls were fastened to the cage door, and towels were provided for bedding. Cages were either barren or equipped with a Hide Perch & Go Box™ [63] of dimensions 46 x 41 x 31 cm, to elicit a broader range of behaviours. Cats were randomly allocated to cages with and without a box, however the final ratio (20:14 respectively) was unequal because 4 cats from the no box group were returned to owners or removed from the study after four days without defecating. Cages were cleaned daily by removing all waste, changing bedding and wiping walls with a clean cloth soaked
in water; and cages were disinfected between cats with a 1% disinfectant solution (Virkon®, Du Pont, Mississauga, Ontario, Canada). Windows provided natural light and an ambient temperature of 20 ±2 °C was maintained. Feed was provided twice daily (at 0700 and 1700 h) (Science Diet, Hill's Pet Nutrition, Inc. ®/™, Vancouver, Canada) and fresh water was provided ad libitum.

2.3 Behavioural Observations

Outside each cage, an infrared camera (Sony CCD25M crystal-View Super Hi-Res ICR IR Camera SLED w/9-22mm Vari-focal Lens, Microtech Advanced Technologies Ltd, Vancouver, Canada) was mounted at cage height on a rod suspended from the ceiling at 1 m from the cage door. Footage was available for viewing real-time in an adjacent room, and was stored for subsequent analysis. The starting point for the construction of indices of wellbeing was an ethogram limited to 37 behaviours reported in observational studies of caged and household cats [3, 64-67]. To facilitate observations, the behaviours were organized into five main categories (location in cage, posture, self-maintenance behaviour, response to humans, and other activities) (Table1). Behaviours were measured as frequency or duration and coded with the aid of Observer software (Noldus Information Technology, Netherland), using focal sampling of 5 min/h.
2.4. S-IgA and cortisol assays

Faecal samples were collected when available (mean =3 stools per cat), weighed and immediately frozen at -40°C. To prepare faecal extracts for IgA ELISA analysis, 10 mL phosphate buffered saline (PBS) containing 10% V/V goat serum (Equitech-Bio) and 0.1 % V/V Kathon (Sigma) was added to 1g wet faeces and then vortexed for 10 minutes or until fully homogenized. A 2 mL aliquot of homogenized sample was centrifuged at 10,000 x g for 10 minutes at 4°C. Complete, EDTA-free Protease Inhibitor cocktail (Roche) was added to aliquots of the supernatant before storage at -80°C. White ELISA plates (Lumitrac 600, Greiner) were coated overnight at 4°C with 100 µL/well goat
anti-cat IgA (Bethyl) diluted to 1 µg/mL in carbonate/bicarbonate coating buffer, pH 9.5. Plates were then blocked for 30 minutes at 37°C with 200 µL/well coating buffer containing nonfat dry milk (5% W/V) and Kathon (0.1% V/V, Sigma), then washed 3x with PBS containing Tween 20 (0.05% V/V) (PBST). Faecal extracts were diluted in PBS containing nonfat dry milk (5% W/V), normal goat serum (20% V/V), Tween 20 (0.05% V/V), and Kathon (0.1% V/V), starting at 1:1000, and extending to 1:128,000. Standards (cat reference serum, Bethyl Laboratories, USA) were diluted to 20 ng/ml, then serially diluted two-fold to 0.31 ng/ml. Diluted samples and standards were added at 100 µL/well in triplicate and incubated at 37°C for 1 hour. Plates were washed five times with PBST, then goat anti-cat IgA peroxidase conjugate (Bethyl), diluted 1:100,000 in sample diluent, was added at 100 µL/well and incubated at 37°C for 60 min. Plates were washed 5x with PBST and developed with 100 µL/well of SuperSignal ELISA Femto Maximum Sensitivity Substrate (Thermo Scientific) for 2 min. They were read immediately in a Wallac Victor³ multilabel plate reader (Perkin Elmer). Prism software (GraphPad) was used to calculate sample IgA concentrations from a standard curve generated by a four-parameter logistic equation.

For faecal cortisol extraction and quantification, 0.2 g faeces were placed into a 15 mL conical centrifuge tube and stored at -80°C. Diluent (5 mL of 90% ethanol in distilled water) was added to thawed faecal samples and then vortexed for 30 minutes. Samples were heated to 98°C for 20 min then centrifuged at 2500 x g for 10 min. Supernatant was collected and analysed using a chemiluminescence assay to measure cortisol in the Immulite 1000 system (Siemens Diagnostics). The assay was validated by spiking blank diluent with known amounts of feline cortisol (United States Pharmacopeia) at low, medium, and high concentrations. In each case the spiked values were within the recommended tolerance range. Results are reported per gram of wet faeces.

2.5 Statistical Analyses

Due to different arrival time of cats on day 1, short periods of camera malfunction, and cats escaping from view, not all cats could be observed for the same allotted time of 24 sessions, each of 300 s, per day. Hence, adjusted daily means were calculated for observation time (duration: s /number of observations) and frequency (frequency: counts*24/number of observation sessions). Observation days not followed by a stool within 48 h were discarded. A total of 1712 sessions, each of 300 s, were observed from days 1-5.
In order to meet the assumptions of principal component analysis (PCA), we reduce the size of the initial set of data. We discarded behaviours with large numbers of zero values (>10 %) when aggregated over the three days. We combined behaviours with seemingly similar motivation and significant correlation to each other (Rs > 0.2, $P \leq 0.05$). We determined the appropriateness of combining the cat groups (box/no box) by Student’s t-test (two tailed) and the data were transformed to Z scores and tested for normality by an Anderson Darling test. To determine change overtime, we performed a GEE and a repeated ANOVA. The mean, sd, se and N for the number of seconds a behaviour was expressed are reported for each of the days.

PCA was applied to the reduced set of data (24 behaviours), and subsequent analyses were performed for each PCA subspace (emotional dimensions). Sampling adequacy was determined for each by the Bartlett test of sphericity and the Kaiser-Meyer-Olkin test.

PCA results were interpreted using biplot analyses [68-70]. According to this methodology, the projection (length) of each vector (line) in the biplot approximates the variances of the variable. How well a variable is represented in each PCA biplot is determined by the coordinates of vector endpoints. Those closer to the parameters of the biplot circle have higher variance and are therefore better represented within the dimension. Very short vectors are considered multivariate outliers. The angle between vectors, more precisely the angle cosine is equal to their Pearson coefficient and therefore indicates the direction and strength of association between variables. Ninety degree angles indicate no correlation ($\cos = 0$). An acute angle indicates a positive correlation, reaching a perfect correlation as the angle became smaller ($\cos = 1$). Obtuse angles signify a negative correlation, reaching a perfect correlation at $180^\circ$ ($\cos = -1$).

The final PCA matrix was processed further by removing all outliers, placing variables in order of importance (from highest to lowest variance), centering the data within the matrix and determining exclusivity of behaviours to each dimension and finally labelling the dimensions according to emotional construct. To verify whether the relationship between each behaviours, cortisol and S-IgA obtained by PCA were significant, a GEE was performed comparing Means for cortisol and S-IgA according to presence or absence of each behaviour.

To uncover clusters of cats best described by each emotional dimension, Z-scores of cats were averaged by dimension and subjected to a PCA. In addition, the characteristics of cats: age
(juvenile, adult, senior), source (owner surrendered, stray), sex (male, female) and sterilization status (neutered, intact) were included in the analysis as supplementary qualitative variables. In this analysis, the distance between observation points approximates the Euclidean distance between observations in the multivariate space.

Mean values of S-IgA and cortisol for each cluster of cats were examined using GEE, which allowed us to account for the correlation between days for each of the cats. A GEE was performed to determine if there was a significant difference in the log IGA and log cortisol levels for sex, source, sterilization status and age.

Analyses were conducted in XLSTAT-Pro 2010 (Microsoft Corp, USA) and R version 2.14.11), using the function geeglm from geepack [71]. Results were considered significant at alpha ≤0.05.

3.0 Results

3.1. Initial reduction of behavioural data

An exploratory Spearman Rank analysis using behaviours with apparently similar motivations and statistically significant correlations (Rs between 0.3 and 0.9, $P \leq 0.05$) resulted in three new behavioural categories (hiding, flat postures and escape bouts). ‘Hiding’ represented the sum of time spent behind the box, in the box but not visible, behind the litter and attempting to hide in the litter tray, or under a towel. The category ‘flat’ represented the sum of time spent in two similar postures (ventral flat and stand flat). ‘Escape bouts’ was constructed by adding frequencies for standing on hind limbs while pawing at the wall/floor or cage door, and upside down posture. To distinguish cats spending time at the front of the cage while trying to escape or while sitting quietly, we constructed two separate categories (front escape and front sit). The behaviours rolled up, on back, circle, hiss, knead, play, and neutral response to a human were discarded due to the large number of zero values (>10%) over the three days of recording. The set of behaviours used for further analyses included the new categories: hide, flat, escape bouts, front escape and front sit, along with those retained from the original ethogram (Table 1): freeze, crawl, walk, pace, push, sleep, rub, scan, startle and meow (activities); eat/drink, groom (self-maintenance behaviours); aggressive, friendly or retreat (responses to humans); and crouch, ventral high, stand tall, lie on side (postures).
Means for cats with and without boxes were compared by Student’s t-test (two-tailed test: \( t (46) =0.68, P=0.50 \)). As no difference was found, the two groups were amalgamated. Data were tested for normality by the Anderson-Darling test (\( A^2 =0.415, P=0.31 \)). Based on GEE and repeated ANOVA, behaviours did not change over days 1, 3 and 5 \( (P \geq0.17) \), with the exception of walk, sleep, friendly to human, and eat/drink, which all increased significantly \( (P<0.05) \) (Table 2).

Table 2: Generalized estimated equation and repeated Anova results for all behaviours coded on days 1, 3 and 5

<table>
<thead>
<tr>
<th>names</th>
<th>p.value GEE</th>
<th>p.value repeated ANOVA</th>
<th>mean</th>
<th>se</th>
<th>N</th>
<th>mean</th>
<th>se</th>
<th>N</th>
<th>mean</th>
<th>se</th>
<th>N</th>
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<td>77</td>
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<tr>
<td>Flat</td>
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<td>0.61</td>
<td>76</td>
<td>79</td>
<td>21</td>
<td>14</td>
<td>49</td>
<td>80</td>
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<td>105</td>
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<td>14</td>
<td>61</td>
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<td>3</td>
<td>7</td>
<td>1.4</td>
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<td>3</td>
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<td>Startle</td>
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<td>0.91</td>
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<td>7</td>
<td>1.9</td>
<td>14</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>24</td>
<td>5</td>
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<tr>
<td>Retreat from H</td>
<td>0.59</td>
<td>0.68</td>
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<td>4</td>
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<td>14</td>
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<td>5</td>
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<td>24</td>
<td>3</td>
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<td>30</td>
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<td>15</td>
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<td>Front escape</td>
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<td>Escape bouts</td>
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<td>29</td>
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<tr>
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<td>0.26</td>
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<td>44</td>
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<td>14</td>
<td>6</td>
<td>14</td>
<td>2.9</td>
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</tr>
<tr>
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<td>0.55</td>
<td>0</td>
<td>1</td>
<td>0.3</td>
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<td>0.32</td>
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<td>19</td>
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<td>26</td>
<td>39</td>
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<td>33</td>
<td>8.8</td>
<td>14</td>
<td>69</td>
<td>61</td>
<td>12</td>
<td>24</td>
<td>62</td>
</tr>
<tr>
<td>Stand tall</td>
<td>0.93</td>
<td>0.96</td>
<td>10</td>
<td>24</td>
<td>6.4</td>
<td>14</td>
<td>8</td>
<td>10</td>
<td>2</td>
<td>24</td>
<td>9</td>
</tr>
<tr>
<td>Lie on side</td>
<td>0.22</td>
<td>0.59</td>
<td>14</td>
<td>35</td>
<td>9.4</td>
<td>14</td>
<td>30</td>
<td>47</td>
<td>9.6</td>
<td>24</td>
<td>27</td>
</tr>
<tr>
<td>Walk</td>
<td>0.00</td>
<td>0.06</td>
<td>0</td>
<td>1</td>
<td>0.3</td>
<td>14</td>
<td>5</td>
<td>6</td>
<td>1.2</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>Sleep</td>
<td>0.02</td>
<td>0.17</td>
<td>24</td>
<td>37</td>
<td>9.9</td>
<td>14</td>
<td>55</td>
<td>49</td>
<td>10</td>
<td>24</td>
<td>52</td>
</tr>
<tr>
<td>Rub</td>
<td>0.22</td>
<td>0.27</td>
<td>0</td>
<td>1</td>
<td>0.3</td>
<td>14</td>
<td>2</td>
<td>4</td>
<td>0.8</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>Friendly to H</td>
<td>0.00</td>
<td>0.01</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>1</td>
<td>2</td>
<td>0.4</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>Eat /drink</td>
<td>0.02</td>
<td>0.10</td>
<td>1</td>
<td>1</td>
<td>0.3</td>
<td>14</td>
<td>1</td>
<td>2</td>
<td>0.4</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>Groom</td>
<td>0.23</td>
<td>0.30</td>
<td>1</td>
<td>2</td>
<td>0.5</td>
<td>14</td>
<td>2</td>
<td>3</td>
<td>0.6</td>
<td>24</td>
<td>2</td>
</tr>
</tbody>
</table>
3.2. Clustering of behaviours

The PCA applied to all behaviours (Matrix 63 observations by 24 behaviours) extracted a three dimensional structure (Fig. 1), indicating the need for three subsequent analyses to obtain a detailed description of the relationship between variables within each dimension.

Fig. 1 PCA biplot of matrix 63 observations days by 24 behaviours showing a three dimensional structure with components Eigen values >1 explaining 65.2% of the variability in the data. Bartlett’s sphericity test (Chi-Sq = 315.8, DF 276, P <0.0001, alpha 0.05) and the Kaiser-Meyer-Olkin test (KMO = 0.75).
The first group included six behaviours (hiding, freeze, flat, startle, crawl and retreat from humans) (Fig. 2). The projection of the vectors (endpoints very close to the circle), indicated a good representation of the six variables within this dimension. Extreme vectors of this group, encompassing all others, formed an acute angle ($\angle 39^0$, $\cos = 0.8$) indicating a strong positive correlation ($P \leq 0.01$). Coordinates of supplementary variables cortisol and S-IgA located in the opposite quadrants indicated their negative association with these behaviours.

Fig. 2 PCA biplot of dimension 1 (matrix of 63 observation days by six behaviours). Bartlett's sphericity test (Chi-Sq = 24.9, DF 15, $P < 0.0001$). Kaiser-Meyer-Olkin measure of sampling adequacy (KMO = 0.85).

The second group included eight behaviours (meow, scan, escape bouts, front escape, aggressive to humans, crouch, pacing and pushing) (Fig. 3). Projection of the vectors revealed a good representation of all variables with the exception of crouch (very short vector). Behaviours meow, scan and escape bouts were strongly associated to each other (vector $\angle 10^0$, $\cos = 0.98$), as were aggressive to humans, pace, and pushing (vector $\angle 30^0$, $\cos = 0.86$). However, extreme vectors of each substructure ($\angle 80^0$, $\cos = 0.1$) showed a very weak association between these two behaviours (meow and aggression), whereas both substructures were strongly associated to

Fig. 3 PCA biplot of dimension 2 (matrix of 63 observation days by eight behaviours). Bartlett's sphericity test (Chi-Sq = 41.3, DF 28, $P < 0.0001$). Kaiser-Meyer-Olkin measure of sampling adequacy (KMO = 0.74).
front escape ($\leq 50^\circ$, cos $\geq 0.6$). The coordinates of S-IgA (in the opposite quadrant to five behaviours) indicated a negative association between S-IgA and the behaviours, whereas cortisol coordinates (same quadrant as five behaviours) indicated a positive association.

The third group included 10 behaviours (lie on side, sleep, ventral high, friendly to humans, walk, eat, groom, rub, stand tall and front sit) (Fig. 4). Representation in order of importance (based on coordinates of vector endpoints) was sleep, lie on side, front sit, groom, walk, eat/drink, friendly to humans, stand tall and rub. Behaviours sleep to front sit ($\leq 40^\circ$, cos =0.8) were strongly correlated with each other, whereas rub and stand tall showed weaker association to the group. Ventral high, a very short vector, was considered an outlier in this dimension. Ventral high was poorly represented (short vector) Coordinates of both S-IgA and S-cortisol (in the same quadrant as the behaviours) indicated a positive association both physiological measures and the behaviours.

Based on the examination of data within each biplot, the PCA correlation matrix which provides the Pearson correlation values between all variables was further processed. We removed behaviours that were poorly represented within a dimension as indicated by short vectors in the biplots (crouch, rub, stand tall and ventral high). Behaviours were placed in order of importance within their respective dimension (from highest to lowest variance determined as by the projection of vectors in the biplots). Data were centered and each dimension was labelled.

As shown in Table 2, dimensions 1, 2 and 3 were labelled Anxiety, Frustration, and Contentment, respectively. Behaviours within the Anxiety and Contentment dimensions showed a strong and significant contrast to each other (R between -0.6 and -0.3, P<0.05); whereas behaviours of the
Frustration and Contentment (R - 0.2 to -0.1, \( P < 0.05 \)) and Frustration and Anxiety (R ≤ -0.3, \( P < 0.05 \)) dimensions were weakly contrasted.

S-cortisol was weakly, but positively, correlated to escape, pace push, aggression (Frustration) and walk, eat and friendly response to a human (Contentment) (R = 0.3, \( P < 0.05 \)). S-cortisol was weakly, but negatively, correlated to flat, hide and freeze (Anxiety) (R between -0.3 and -0.2, \( P < 0.05 \)); but not significantly correlated to startle, crawl and retreat (Anxiety) (R = - 0.1, \( P < 0.05 \)).

S-IgA was strongly and positively correlated to all Contentment behaviours (R ≥ 0.6, \( P < 0.05 \)), whereas it was strongly and negatively correlated to Frustration (R between -0.3 and -0.2, \( P < 0.05 \)) and Anxiety behaviours (R between -0.4 and -0.5, \( P < 0.05 \)).

A GEE analysis confirmed that Loge cortisol was significantly greater (\( P < 0.05 \)) for Frustration behaviours: Pace, Pushing, Redirected aggression when present (Mean 1.44 µg/g) versus absent (Mean 1.19 µg/g). Similarly, Loge cortisol was greater when Contentment behaviours: Walking and Friendly to humans were present (Mean 1.30 µg/g) versus absent (Mean 1.20 µg/g). There was a trend (\( P = 0.06 \)) for Loge cortisol to be higher when Escape behaviours were present (Mean 1.33 µg/g) versus absent (Mean 1.21 µg/g). Conversely, Loge cortisol was significant lower when Anxiety behaviour Freeze was present (Mean 1.13 µg/g) versus absent (Mean 1.42 µg/g). S-IgA Means were significantly different for all behaviours (absent versus present) (\( P < 0.02 \)) with the exception of Scan (\( P < 0.07 \)).
Table 3: Scaled PCA Pearson correlation matrix

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1: Anxiety</th>
<th>Group 2: Frustration</th>
<th>Group 3: Contentment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flat Freeze Hiding Crawl Startle H</td>
<td>Esc bouts Scan Front esc. Pushing Pace Aggress H</td>
<td>Sleep On side Frontal Groom Walk Eat/drink Friendly H S-IgA Cortisol</td>
</tr>
<tr>
<td>Flat</td>
<td>1 0.8 0.9 0.7 0.7 0.7</td>
<td>-0.2 -0.2 -0.1 -0.3 -0.2 -0.3 -0.1</td>
<td>-0.5 -0.4 -0.4 -0.3 -0.4 -0.3 -0.4</td>
</tr>
<tr>
<td>Freeze</td>
<td>0.8 1 0.8 0.7 0.8 0.7</td>
<td>-0.3 -0.3 -0.1 -0.3 -0.3 -0.3 -0.1</td>
<td>-0.5 -0.5 -0.4 -0.5 -0.4 -0.4 -0.4</td>
</tr>
<tr>
<td>Hiding</td>
<td>0.9 0.8 1 0.7 0.7 0.8</td>
<td>-0.2 -0.2 -0.1 -0.3 -0.2 -0.3 -0.1</td>
<td>-0.6 -0.4 -0.4 -0.5 -0.4 -0.4 -0.3</td>
</tr>
<tr>
<td>Crawl</td>
<td>0.7 0.7 0.7 1 0.7 0.8</td>
<td>-0.2 -0.2 0.0 -0.2 -0.1 -0.2 -0.1</td>
<td>-0.4 -0.3 -0.3 -0.4 -0.2 -0.3 -0.3</td>
</tr>
<tr>
<td>Startle</td>
<td>0.7 0.8 0.7 0.7 1 0.9</td>
<td>-0.2 -0.2 0.0 -0.2 -0.2 -0.2 0.0</td>
<td>-0.5 -0.4 -0.4 -0.4 -0.4 -0.4 -0.4</td>
</tr>
<tr>
<td>Retreat H</td>
<td>0.7 0.7 0.8 0.8 0.9 1</td>
<td>-0.2 -0.2 0.0 -0.2 -0.2 -0.2 0.0</td>
<td>-0.5 -0.4 -0.3 -0.4 -0.3 -0.4 -0.4</td>
</tr>
<tr>
<td>Meow</td>
<td>-0.2 -0.3 -0.2 -0.2 -0.2 -0.2</td>
<td>1 0.9 0.8 0.6 0.5 0.5 0.0</td>
<td>-0.2 -0.2 -0.2 -0.1 -0.1 -0.2 -0.1</td>
</tr>
<tr>
<td>Esc bouts</td>
<td>-0.2 -0.3 -0.2 -0.2 -0.2 -0.2</td>
<td>0.9 1 0.8 0.8 0.6 0.6 0.1</td>
<td>-0.2 -0.2 -0.1 -0.1 -0.1 -0.1 0.0</td>
</tr>
<tr>
<td>Scan</td>
<td>-0.1 -0.1 -0.1 0.0 0.0 0.0 0.0</td>
<td>0.8 0.8 1 0.7 0.6 0.5 0.2</td>
<td>-0.3 -0.2 -0.2 -0.2 -0.2 -0.2 -0.2</td>
</tr>
<tr>
<td>Front esc.</td>
<td>-0.3 -0.3 -0.3 -0.2 -0.2 -0.2</td>
<td>0.6 0.8 0.7 1 0.8 0.8 0.6</td>
<td>-0.2 -0.2 -0.1 -0.2 -0.1 -0.1 -0.1</td>
</tr>
<tr>
<td>Pushing</td>
<td>-0.2 -0.3 -0.2 -0.1 -0.2 -0.2</td>
<td>0.5 0.5 0.6 0.8 0.1 0.9 0.7</td>
<td>-0.2 -0.2 -0.2 -0.2 -0.1 -0.1 -0.1</td>
</tr>
<tr>
<td>Pace</td>
<td>-0.3 -0.3 -0.3 -0.2 -0.2 -0.2</td>
<td>0.5 0.5 0.5 0.5 0.9 1 0.8</td>
<td>-0.2 -0.2 -0.1 -0.2 -0.1 -0.1 -0.1</td>
</tr>
<tr>
<td>Aggress H</td>
<td>-0.1 -0.1 -0.1 -0.1 0.0 0.0 0.0</td>
<td>0.0 0.1 0.2 0.6 0.7 0.8 1</td>
<td>-0.1 -0.1 -0.1 -0.2 -0.1 -0.1 -0.2</td>
</tr>
<tr>
<td>Sleep</td>
<td>-0.5 -0.5 -0.6 -0.4 -0.5 -0.5</td>
<td>-0.2 -0.2 -0.3 -0.2 -0.2 -0.2 -0.1</td>
<td>1 0.8 0.7 0.6 0.7 0.6 0.6</td>
</tr>
<tr>
<td>On side</td>
<td>-0.4 -0.5 -0.4 -0.3 -0.4 -0.4</td>
<td>-0.2 -0.2 -0.2 -0.2 -0.2 -0.2 -0.1</td>
<td>0.8 1 0.7 0.6 0.7 0.6 0.5</td>
</tr>
<tr>
<td>Frontal</td>
<td>-0.4 -0.4 -0.4 -0.3 -0.4 -0.3</td>
<td>-0.2 -0.1 -0.2 -0.1 -0.2 -0.1 -0.1</td>
<td>0.7 0.7 1 0.6 0.6 0.7 0.5</td>
</tr>
<tr>
<td>Groom</td>
<td>-0.5 -0.5 -0.5 -0.4 -0.4 -0.4</td>
<td>-0.1 -0.1 -0.2 -0.2 -0.2 -0.2 -0.2</td>
<td>0.6 0.6 0.6 1 0.6 0.7 0.5</td>
</tr>
<tr>
<td>Walk</td>
<td>-0.4 -0.4 -0.4 -0.2 -0.4 -0.3</td>
<td>-0.1 -0.1 -0.2 -0.1 -0.1 -0.1 -0.1</td>
<td>0.7 0.7 0.6 0.6 1 0.7 0.5</td>
</tr>
<tr>
<td>Eat/drink</td>
<td>-0.3 -0.4 -0.4 -0.3 -0.4 -0.4</td>
<td>-0.2 -0.1 -0.2 -0.1 -0.1 -0.1 -0.1</td>
<td>0.6 0.6 0.7 0.7 1 0.5</td>
</tr>
<tr>
<td>Friendly H</td>
<td>-0.4 -0.4 -0.3 -0.3 -0.4 -0.4</td>
<td>-0.1 0.0 -0.2 -0.1 -0.1 -0.1 -0.2</td>
<td>0.6 0.5 0.5 0.5 0.5 0.5 1</td>
</tr>
<tr>
<td>S-IgA</td>
<td>-0.4 -0.5 -0.4 -0.3 -0.4 -0.4</td>
<td>-0.2 -0.2 -0.3 -0.3 -0.2 -0.2 -0.2</td>
<td>0.8 0.8 0.7 0.6 0.7 0.6 0.8</td>
</tr>
<tr>
<td>Cortisol</td>
<td>-0.2 -0.3 -0.2 -0.1 -0.1 -0.1</td>
<td>0.1 0.2 0.0 0.3 0.2 0.3 0.2</td>
<td>0.1 0.0 0.0 0.0 0.3 0.2 0.3</td>
</tr>
</tbody>
</table>
3.3 Clustering of observations

The averaged Z scores subjected to PCA revealed the formation of 3 distinct clusters (Fig. 5). Over all days, 44% of observations clustered with Anxiety, compared with 19% and 37% with the Frustration and Contentment dimensions, respectively. The characteristics owned, senior and spayed female clustered in the Anxiety dimension. Adult, stray and intact male clustered in the Frustration dimension and juvenile and intact female clustered in the Contentment dimension.

![PCA biplot of observations](image)

Fig.5 PCA biplot of observations (Matrix: 63 observation days by three variables) extracted two components with Eigen values >1 explaining 90% of the variability in the data. Measures of adequacy were by Bartlett's sphericity test (Chi-Sq = 223.16, DF 190, P < 0.001) and Kaiser-Meyer-Olkin measure of sampling adequacy (KMO = 0.80). Neut male = neutered male, Sp. Female = spayed female.
3.4. S-IgA and cortisol

Intra-assay coefficients of variability (CVs) were 5.4% and 7.1% for s-IgA and cortisol, respectively. S-IgA and cortisol inter-assay CVs were 9.1% and 7.9%, respectively. There was no significant correlation between S-IgA and cortisol (GEE, \( P = 0.37 \)), and no significant difference overtime for either S-IgA (GEE, \( P = 0.80 \)) or cortisol (GEE, \( P = 0.57 \)). A comparison of the first day with the other days using a t-test found no significant difference for S-IgA (\( P = 0.11 \)) or cortisol (\( P = 0.15 \)).

3.5 Behaviours, Emotions, S-IgA and cortisol

As shown in Table 4, Log\(_e\) transformed S-IgA values were significantly greater (\( P < 0.001 \)) for Contented cats (mean 7.1 ±0.5 log\(_e\) µg/g), compared to the Anxious (5.6 ±0.6 log\(_e\) µg/g), and Frustrated (5.6 ±0.7 log\(_e\) µg/g) cats. However, S-IgA concentrations of Frustrated and Anxious cats did not differ (\( P > 0.05 \)). Cortisol values were not significantly different for Frustrated (4.2 ±2.0 log\(_e\) µg/g), Content (3.7 ±1.6 log\(_e\) µg/g), and Anxious cats (3.0 ±1.0 log\(_e\) µg/g) (Estimate 0.13, SD 0.31, Wald 1.67E-01, \( P = 0.68 \)). There was no significant difference in cortisol and in S-IgA according to sex, source, neuter status, and age (\( P > 0.05 \), GEE analysis).

<table>
<thead>
<tr>
<th>Table 4 Generalised estimating equation for S-IgA by cat clusters</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image.png" alt="Table" /></td>
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</tbody>
</table>

4.0 Discussion

A multidimensional model of emotional states in shelter cats was constructed as a basis for indices of wellbeing suitable for the assessment of emotions and susceptibility to upper respiratory disease
Chapter 2

(URD) in shelter cats. We examined the behavioural responses of cats and their physiological correlates, more specifically secretory immunoglobulin A (S-IgA) and cortisol. Composite indices of wellbeing we called the University of Queensland Indices of Cat Wellbeing (UQ-ICW) were constructed in several stages. We first selected 37 behaviours used in other studies for the assessment of welfare in shelter and household cats. Post assessment of the cats, the data set was reduced by amalgamating various behaviours with seemingly similar motivation and significant Spearman correlations. Others were removed as they were unfrequently observed. An initial principal component analysis (PCA) was applied to the remaining 24 behaviour variables producing a three dimensional graphical display of the data. The internal structure of behavioural within each dimension was further examined together with physiological measures and processed using biplot analysis methodology proposed by Gabriel [68]. The resulting matrix (UQ-ICW) was used to uncover clusters of cats that may be best represented in each dimension. Z scores for each cats (representing total time spent engaged in a behaviour per day) were averaged for each dimension and analysed together with characteristics such as age (juvenile, adult, senior) , sex and sterilization status and source (stray versus owner-surrendered). The significance of the correlations uncovered by PCA between the behaviour variables and physiological measures and subsequently between clusters of cats and physiological measures were corroborated by generalised estimating equations (GEE).

Although there is a vast literature describing emotion-related behaviours in cats when subjected to brain stimulation, psychoactive drugs or other forms of invasive procedures, to our knowledge this is the first attempt to qualify emotional arousal in shelter cats using non-invasive measures of behaviour and their physiological correlates. While we cannot and do not claim that we have measured emotions directly, our interpretation of findings are consistent with motivational theory of emotions and consistent with detailed descriptions of emotion-specific behaviours of cats described in the affective neuroscience literature.

The dimension labelled Anxiety included six strongly correlated defensive behaviours believed to be characteristic of high arousal of the emotional defence system. These behaviours were consistent over the five days of observation indicating that this emotional state was chronic. For the most part, cats remained at the back of the cage immobile while maintaining a flat posture. They attempted to hide behind or in the litter tray, under the bedding or the box. Locomotion was inhibited, when it occurred it was slow and accompanied by low posture (crawling). We observed frequent startle response to outdoor sounds such as barking dogs or when people entered the room. When approached or during regular cleaning of the cage, the cats slowing retreated against the back wall, further flattened their body (including the ears). Sleeping, eating, and grooming were also inhibited particularly in the presence of humans. While these defensive behaviours are commonly interpreted as fear in shelter cats
[3,72], the absence of aggressive behaviours such as hissing, growling or striking at staff during the cleaning of their cage supports its interpretation as Anxiety. The absence of fear response in our cats may have been due to our hands-off protocol. At no time after being placed in the cage was there an attempt to touch the cats. The neuroscience literature is consistent in differentiating defensive attack (fear) and defensive retreat (anxiety) in cats. Firstly, these two emotional responses have distinct neural correlates. Defensive attack and defensive retreat are produced by the stimulation of the dorsal and ventral part of the ventromedial hypothalamus (VMH), respectively [31]. The authors also found that defensive retreat (chronic state) had immunosuppressive effect whereas defensive attack (acute response) did not inhibit the immune response of cats when injected with a toxin [34, 35]. Similarly, the ethological literature differentiates these states. Anxiety is described as a state elicited by uncertainty about potential risks, usually when faced with novel situations. It elicits hyper-vigilance (orienting attention towards the stimulus) and inhibition of normal activities such as eating, grooming, sleeping, and locomotion to facilitate appraisal of the threat. On the other hand, fear is elicited when the stimulus has been appraised as posing a real risk thus it is marked by flight, or defensive attack [33]. In cats (when escape is not possible), fear is characterized by hissing, flattening of the ears, drawing in of the head, piloerection, stiffening of the tail, and paw striking [73] with low intensity growl and hiss usually occurring just prior to attack [74]. The distinction between anxiety and fear has important implications for the welfare of cats. In animal shelters, fearful cats are often classified as feral or non-adoptable due to aggression and subsequently euthanized (Personal communication March 2011, Dr. Jamie Lawson, British Columbia Society for the Prevention of Cruelty to Animals).

We had expected to find elevated cortisol in cats rated as Anxious as they are usually considered to be highly stressed. However, flat and hide showed a negative trend to cortisol (non-significant) and freeze was significantly and negatively correlated to cortisol. In accordance with our findings, hiding specifically has been associated with low cortisol in laboratory cats [67]. The authors speculated that hiding and associated behaviours (e.g. flattened posture) may help cats self-manage their stress resulting in a reduction in adrenal output. While this is a likely explanation, the inhibitory effect of anxiety on the central nervous system may have been mediated by an increase in glutamate. The neuropeptide glutamate which plays an important role in modulating anxiety is known to have an inhibitory effect on the production of stress hormones in various species [75, 76]. Further research may elucidate the glutamate-cortisol relationship in cats under stressful conditions. The current findings contribute to the literature highlighting the complexity of the relationship between chronic psychological states and hypothalamic-pituitary-adrenal activation [77]. Studies that qualify emotional arousal together with measure of S-IgA in animals are sparse. Nonetheless, the inhibition of S-IgA found in cats labelled as Anxious in this study is consistent with studies in humans. For example, nurses reporting more frequent episodes of anxiety have significantly lower S-IgA than nurses reporting only occasional episodes of anxiety [78]. A decreased in anxiety following a therapeutic massage
was found to coincide with an increase in S-IgA [79]. Although one study reported no changes in S-IgA in women provided with relaxation therapy to reduce anxiety [80].

In contrast to cats clustered in the Anxiety dimension, those clustered in the Contentment dimension sat calmly at the front of the cage watching activities or resting/sleeping in a relaxed position (lying on their side). They groomed, they ate, and they rubbed on objects in the cage. When walking, they were upright and moved confidently and calmly around the cage. When approached, they responded in a friendly manner. Mendl and colleagues [20] have proposed that calm / relaxed states are characteristic of low arousal of the emotional defence system. This notion of anxiety and contentment as opposite states of the same motivational dimension was supported by our graphical display of these two groups of behaviours. In our PCA biplot (Fig. 1) representing the three emotional states, the Anxiety and Contentment dimensions were clearly contrasted (opposite quadrant). Further, the PCA Pearson correlation matrix showed significantly negative correlations between Anxiety and Contentment behaviours further suggesting that they each represent opposite ends (low & high arousal) of the defence response to threat. We therefore interpreted behaviours of the Contentment behaviour as a state of emotional wellbeing (positive affect). There is agreement across studies that healthy well-adjusted adult cats spend 16% of their sleep time in rapid eye movement sleep; with postural indicators such as lying on their side, with the head resting on forepaws or floor, and relaxed neck muscle tonus [81-83]. Conversely, cats that are stressed by a change in light/dark ratio were found to significantly inhibit deep sleep [84]. In this study, cats considered Content showed postural characteristics of deep sleep (lying on side), suggesting that they were not negatively affected by the shelter conditions. Nor did they spend more time sleeping than the expected range or engage in feigned sleep (also called defensive or tense sleep and indicated by pressed eyes and tense posture), both of which have been associated with depression and psychological stress, respectively, in shelter cats [3, 85].

Hypothalamic stimulation, that elicits increased vocalizations and defensive postures, has been associated with both cessation of grooming and excessive perineal licking [30]. This suggests an affective component of grooming behaviour. Similarly, excessive grooming behaviour [86], hair pulling (psychogenic alopecia) [87], and cessation of grooming are believed to be indicators of anxiety, boredom, and unfulfilled desire for human contact [88]. The Content cats in this study engaged in normal pattern of grooming further supporting the notion of wellbeing [89]. Sitting at the front of the cage observing activities is a common behaviour in domestic cats. Bernstein and Strack [90] found that household cats (presumed to have good welfare) can spend up to 5 hours per day watching activities from a windowsill. Indeed, it has been recommended that shelter cats be provided with perches specifically to facilitate this “natural” behaviour with the aim of improving
their welfare [91]. Although, cortisol values were not significantly different between the clusters of cats, three behaviours from this group (eat, walk and friendly) were positively correlated to cortisol. While, we had not expected to find elevated cortisol associated with emotional wellbeing (positive affect), these findings are not contradictory to concepts of stress. Stress is a physiological response to any change or demand on the body whether it is positive, negative or neutral [92]. For example, the behaviour “friendly” involved the cat rising and approaching the human. In humans, the simple process of awakening and getting out of bed can increase cortisol [93]. The anticipation of food can increase cortisol with level remaining high during feeding [94].

Thus the positive relationship between cortisol and these behaviours was likely due to a change and increase in activity level and were not interpreted as indicators of emotional stress.

The hypothesis that positive affect may stimulate S-IgA in animals [95] as it does in humans [96] was confirmed in this study. All Contentment behaviours were significantly and positively correlated to S-IgA and Content cats had a mean S-IgA value significantly higher than that of other cats. The clinical relevance of different levels of S-IgA to URD has not been determined, further research is needed to determine if the levels reached by Content cats in this study may be sufficient to protect them against opportunistic URD pathogens in the shelter environment.

The third dimension included behaviours believed to be characteristic of frustration. This emotional dimension was characterized by persistent meowing and visual scanning of the cage. Frustrated cats paced, pushed items around the cage, stood on hind limbs while pawing at walls, spread litter and food around the cage and destroyed bedding. They spent much time at the front of the cage attempting to open the latch, putting their paws through the bars or pushing on the front of the door with their head or body and hanging upside down on the door. Some cats showed short burst of redirected aggression that were not associated with defensive behaviours observed in the Anxious cats. The aggression observed in these cats was more characteristic of redirected aggression described by Beaver [97] in cases when a housebound cat views another cat through a window and subsequently attacks any available target in the house (people or other pets). Frustration behaviours in this study were qualitatively similar to a group of behaviours labelled restlessness elicited by in brain stimulation [98]. The authors describe restlessness as arousal followed by walking restlessly in the cage, meowing, peeping and inserting a paw into a crevice of the cage and escaping if the door was opened together with indicators of stress such as pupil dilation and increased heart rate. The authors had concluded that these behaviours may be representative of arousal of the defence system. The interpretation was, however, not congruent with our PCA results which revealed a weak and non-
significant contrast between Frustration and the behaviours in both the Anxiety and Contentment dimensions. Our interpretation supports notions proposed by motivational theorists [18, 99] that this group of behaviour represents the behavioural repertoire associated with the reward (appetitive) system. The reward (appetitive) system modulates emotions which range from frustration (low arousal) to pleasure (high arousal). Frustration is known to occur under uncontrollable conditions, such as delay or omission of an expected reward (e.g. food) [100]; and thwarting of ethological needs, such as the inability to perform species-specific behaviours [101]. Stereotypic grooming, vocalization, rocking, pacing and self-mutilation have also been observed in laboratory cats in response to unpredictable husbandry [102] and are characteristic of frustration in other captive carnivores [103, 104]. In accordance with other studies [105-107], cortisol was positively correlated to pacing, pushing, escape behaviours and aggression. However, this was not sufficient to cause a significant difference in Mean cortisol between the clusters of cats. Although, this may have been due to the small number of cats providing data for this emotional state (n=9). Further research on the frustration –cortisol link in shelter cats is needed. Research relating frustration to fluctuation in S-IgA is sparse. However, we found a similar inhibition of S-IgA in the Frustrated cats as those reported in humans asked to recall an event that had made them frustrated [108].

Overall the findings in this study emphasize the importance of determining the qualitative nature of emotional arousal [109, 110] as they are differently related to mucosal immunity with possible implication for health (particularly susceptibility to upper respiratory disease). However, important limitations of this study should be noted, in addition to the most obvious one regarding sample size (n=34). Graphical interpretation of multivariate relationships between variables, widely used in some disciplines (i.e. genetics, biology) [111], has not been previously applied to the study of animal emotions. Thus, there may be issues with this approach that affected results. For example, contrary to recommendations for traditional PCA analysis, biplot methodology does not involve the use of orthogonal rotations (e.g., Varimax), thus none were applied to this data. Also, there are various statistical options for the construction of multidimensional models. Some authors recommend the use of Generalised Canonical Correlation Analysis (CCA) rather than PCA to construct biplots [112]. Further research may be necessary to compare the robustness of various approaches. Despite the possible issues highlighted, the use of PCA biplot analysis to construct multidimensional models of wellbeing appears promising.
A limitation regarding the interpretation of S-IgA results must also be highlighted. Some of the cats believed to be anxious did not produce a stool for several days following admission to the shelter, which led to the removal of many observation days that likely coincided with strong emotional arousal. This may have influenced results. Furthermore, our decision to quantify S-IgA from stools produced within 24 to 48 h post observation was selected according to the known time course between a stress event and the appearance of cortisol metabolites in faeces [113, 114] which may not be similarly appropriate for S-IgA. Further research is needed to establish the time course between emotional arousal and changes in concentration of S-IgA in stools. Moreover, whilst the S-IgA inter-intra assay variability (≤ 10%) had clinical relevance [115], there is still no single recognised method to quantify the production of antigen-specific IgA at mucosal surfaces because of the difficulty in normalizing a measured concentration to a standard (wet or dry faecal weight). However, S-IgA is produced to protect the mucosal surfaces of cats from pathogens, and antibody concentration is without a doubt an important part of its efficacy. Despite the potential variability in S-IgA, temporally or due to diet, which would influence faecal composition, the concentrations produced strong correlations to emotions. These issues merit further study and interpretation must take into consideration the caveats of measuring faecal antibodies. Despite these limitations, the UQ-ICW- a behavioural tool for the assessment of emotional states related to mucosal immunity appears promising.

5.0 Conclusions

Three emotional domains were identified in shelter cats, corresponding to motivational constructs of anxiety, frustration and contentment. Anxious and frustrated cats had significantly lower faecal S-IgA concentrations than Content cats. Although significantly correlated to specific behaviours, mean cortisol levels were not significantly different between the Anxious, Frustrated and Content cats.

References

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11. Buffington CAT: Pandora syndrome: rethinking our approach to idiopathic cystitis in cats; cats with idiopathic cystitis often have multisystemic comorbidities, so look outside the urinary tract--as well as in the cats’ environment--for diagnostic and therapeutic answers. JAVMA-J Am Vet Med Assoc 2011, 220(7):994-1002.


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Chapter 3: Effect of gentling on behaviour, mucosal immunity and upper respiratory disease in anxious shelter cats.

Abstract

Emotional, behavioural, and health benefits of gentle touch have been documented for several species, but little is known about the effect of Gentling on cats in stressful situations. In this study, 139 cats rated as Anxious upon admission to an animal shelter were allocated to either a Gentled or Control group. Cats were gentled four times daily for 10 mins over a period of 10 days, with the aid of a tool for cats that were too aggressive to handle. Emotional state was rated daily for 10 d as Anxious, Frustrated or Content using the Queensland Indices of Cat Wellbeing. Gentled cats were less likely to have negatively valenced emotional states (Anxious or Frustrated) (Incidence Ratio [IRR] =0.61 CI 0.42-0.88, \( P =0.007 \)) and more likely to be rated as content compared to control cats (IRR: 1.52, CI: 1.27-1.81, \( P <0.0001 \)). Total faecal IgA was quantified by enzyme-linked immunosorbent assay. Gentled cats had increased S-IgA (6.9 ±0.7 loge µg/g) compared to control cats (5.9 ±0.5 loge µg/g) (\( P <0.0001 \)). Within the Gentled group of cats S-IgA values were higher for cats that responded positively to gentling (7.03 ±0.6, loge µg/g), compared with those that responded negatively (6.14 ±0.8, loge µg/g). Combined conjunctival and oropharyngeal swab specimens were tested by quantitative real-time PCR for feline herpesvirus type 1 (FHV-1), feline calicivirus (FCV), Mycoplasma felis, Chlamydophila felis, and Bordetella bronchiseptica. There was a significant increase in shedding over time in non-gentled cats (23%, 35%, 52% on days 1, 4 and 10, respectively), but not gentled cats (32%, 26%, 30% on days 1, 4 and 10, respectively) (GEE \( P =0.001 \)). Onset of upper respiratory disease was determined by veterinary staff based on clinical signs such as ocular or nasal discharge. Control cats were 2.4 (CI: 1.35-4.15) times more likely to develop upper respiratory disease over time than gentled cats (\( P <0.0001 \)). It is concluded that gentling anxious cats in animal shelters can induce positive affect (contentment), increase production of S-IgA, and reduce the incidence of upper respiratory disease.

Keywords: Emotions, Gentling, Respiratory disease, Secretory Immunoglobulin A, Shelter cats.
1.0 Introduction

In humans, the relationship between negative life events and susceptibility to diseases, such as the common cold, is well established [1-3]. In cats too, a stressful event, such as entering an animal shelter, can reactivate subclinical conditions (e.g. feline herpesvirus type 1), resulting in the onset of clinical upper respiratory disease (URD) [4]. In humans and some other animal species, emotional stress inhibits the production of mucosal antibodies, particularly secretory immunoglobulin A, resulting in increased susceptibility to pathogens that cause URD. Hence, the management of emotional stress may be of clinical importance in managing respiratory disease [5-8].

Physical contact between cats, such as allogrooming (grooming each other) and allorubbing (rubbing on each other), facilitates social bonding [9, 10]; and petting seems to serve a similar purpose in the cat/human relationship [11]. In the home, interactions between cats and owners tend to be characterized by frequent physical contact, such as petting, lifting and holding. In addition, both cats and people seek this physical contact [12]. Physical contact with humans has been reported to increase emotional wellbeing in various domestic species. Laboratory cats show a preference for human interaction over toys [13]. Petting can reduce the heart rate in dogs [14] and horses [15]; and reduce fear of humans in cows [16], rabbits [17] and dogs [18-21]. Petting and therapeutic massage of cats are believed to reduce stress associated with chronic pain [22], and five min of petting can reduce arterial blood pressure [23]. Conversely, cessation of petting has been associated with an increase in the level of cortisol in laboratory cats accustomed to receiving petting during routine care [24].

Despite the documented benefits, in some cats even gentle petting may induce aggression [25]. This is marked by restlessness, tail twitching, increased muscle tension, leaning away, flattened ears, horizontal retraction of the lips, and hissing [26]. It has been suggested that the epidermal units (Merkel cells, Ruffian endings and vibrissae) of cats discharge rapidly, making them highly sensitive to touch, particularly when under stress [25]. In addition, approximately 20% of cats are thought to be genetically predisposed towards defensive behaviour to humans, which is not affected by prolonged socialization [27-29]. Thus, tactile enrichment, such as petting, gentling or massage, can be expected to fail in some cats; particularly those with a timid temperament or when poorly socialized to humans, and under stress. However, petting in the temporal region (between the eyes and ears) rather than in the caudal region [30], and delivery using short strokes with circular movements [31], may reduce such negative responses.

In various species, gentle stroking has successfully reduced the immunosuppressive effects of various husbandry practices. For example, under artificial rearing conditions, lambs usually experience a decrease in secretory immunoglobulin A (S-IgA), which is prevented by providing
gentling [32]. S-IgA is the most abundant mucosal antibody and is necessary for protection against pathogens in the environment that can be inhaled or ingested [33]. The importance of mucosal immunity is well documented in cats, and stimulation of S-IgA is the main goal in the development of effective intranasal vaccines to protect cats against URD pathogens [34, 35]. Emerging attitudes in veterinary medicine emphasize the importance of addressing negative emotional states in animals, as they may be compromise health [6, 8]. The hypothesis examined in this study was that suitable gentling of cats in a shelter would reduce negative emotional arousal and increase S-IgA with a concomitant reduction in URD.

2.0 Material and methods

This study was approved by the University of Queensland Animal Ethics Committee (CAWE/231/10).

2.1 The housing facility and behaviour observation equipment

The study took place at the Vancouver Branch of the British Columbia Society for the Prevention of Cruelty to Animals (BC SPCA, Vancouver, Canada). The shelter had six separate housing areas, with a maximum capacity to house 120 cats. The facility also included an isolation area for sick cats and an on-site veterinary hospital. A small room adjacent to the reception area was used for examination and vaccination of incoming cats.

The facility was maintained at a constant temperature of 20 ± 2 °C, and was naturally lit with the addition of artificial light provided for 4 h each day. Visitors were discouraged from entering the experimental ward; however, approximately 24 people over the course of the study were provided entry to look for their stray cats. Apart from this, the only people entering the ward were shelter staff and two research staff. In common with most shelter environments, some sounds of dogs barking, and people walking and talking nearby, were audible to the human hear.

The housing facility included an associated food preparation area out of sight of the cats. Feed was provided twice daily at 0700 and 1700 h and comprised of 70 g of age-appropriate pellets and approximately 30g of wet food (Science Diet, Hill's Pet Nutrition, Inc. ®/™ Topeka, KS, USA). Fresh water was provided ad libitum. Feeding was undertaken by the experimenter, shelter staff or volunteers.

Twenty stainless steel cages (76 x 76 x 71 cm) were utilised for the study. Each was furnished with litter boxes and non-absorbent cat litter (Veterinary Concepts, Wisconsin, USA), a stainless steel food and water bowl, and a towel for bedding. Outside each cage, an infrared camera (Sony CCD25M crystal-View Super Hi-Res ICR IR Camera SLED w/9-22mm Vari-focal Lens, Microtech...
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Advanced Technologies Ltd, Vancouver, Canada) was mounted at cage height on a rod suspended from the ceiling at 1 m from the cage door. Footage was available for viewing real-time in an adjacent room, and was stored for subsequent analysis.

2.2 Biosecurity

The shelter staff cleaned cages daily by removing all waste, changing bedding, and wiping walls with a clean cloth soaked in water. Cages were disinfected between cats with a 1% disinfectant solution (Virkon®, Du Pont, Mississauga, Ontario, Canada). Staff and the experimenter sanitized their hands (Microsan™ Antiseptic instant hand sanitizer. DEB Worldwide Healthcare Inc. Ontario, Canada) following contact with a cat. Cats with observed clinical signs of URD, such as ocular or nasal discharge, were examined by medical staff and sent to a medical isolation ward if clinical URD was confirmed.

2.3 Animals

This study was part of a research project designed to examine the effects of behavioural interventions on cats rated as Anxious, Frustrated or Content upon admission. Cats were either surrendered by their owner, or brought in as strays by a humane officer, between May and November 2010. Age was either provided by the owners or estimated by shelter staff. Cats were categorized as juvenile (6-12 months), adult (1-7 years) or senior (>8 years). Cats were examined upon admission by an animal health technician, vaccinated (Fel-O-Guard+3 Boehringer Ingelheim Ltd., Burlington, Ontario, Canada) and dewormed (Strongid® T. Pfizer, Quebec, Canada). Combined conjunctival and oropharyngeal swab specimens were collected before vaccination during the initial examination and on days 4 and 10. Samples were tested by quantitative real-time PCR [36] for feline herpesvirus type 1 (FHV-1), feline calicivirus (FCV), Mycoplasma felis, Chlamydophila felis, and Bordetella bronchiseptica. Following examination, cats were transported by staff in a small wire cage covered with a towel to the study ward, a journey of 2 min which did not require passing through any other cat housing units or dog areas. Staff lifted each cat into their cage (covered with a towel prior to lifting if they were growling or hissing) and immediately exited the room.

2.4 Behavioural observation upon admission (day 0)

A 1 h real-time video observation commenced as soon as the cat was placed in the cage. This was followed by a Human-Approach Test, adapted from Kessler and Turner [37] as follows: Step 1: the experimenter stood in front of the cage without interaction, such as eye contact or verbal greeting (2 min); Step 2: the experimenter talked to the cat using a high-pitched gentle tone, and had some eye
contact, but with eyes half closed) (1 min); Step 3: the same procedure was repeated with the door open, followed by an approach of the hand so that it was near the cat (2 min). If the cat attempted to attack (bite or paw strike with hiss and/or growl) the door was closed immediately. Following the observation period and the approach test, cats were assigned an emotional rating of Anxious, Frustrated or Content.

Cats received a rating of Anxious at intake (day 0) if:
1) They had remained at the back of the cage in a flattened posture during the entire observation period.
2) They had attempted to hide under bedding or in the litter box
3) They had retreated or further flattened their body when approached during the approach test or responded with defensive aggression (hiss, growl, paw strike)

Of the 250 cats assessed upon admission, 139 cats were rated as Anxious and enrolled in this study. They were alternately allocated to either a Gentling Treatment group or a Control group.

2.5 Daily rating of emotional states (Mood)

Behavioural tool

Mood per day for each cat was rated using behavioural indicators according to the University of Queensland Indices of Cat Wellbeing-UQ-ICW (a behavioural tool for the assessment of emotional states that are related to mucosal immunity) developed in our previous study. In brief, UQ-ICW was developed from examination of behavioural responses and physiological correlates, more specifically secretory immunoglobulin A (S-IgA) and cortisol in 34 cats during their first week at an animal shelter. UQ-ICW was constructed in several stages. We first selected 37 behaviours used in other studies for the assessment of welfare in shelter and household cats. Each of these states were found to be chronic in cats during their first week at the shelter (lasting 5 days) with the exception of 4 behaviours (walk, eat, sleep and friendly) which were part of the Contentment dimension. Post assessment of the cats, the data set was reduced by amalgamating various behaviours with seemingly similar motivation and significant Spearman correlations. Others were removed as they were unfrequently observed. The 24 remaining variables were subjected to a principal component analysis producing a three dimensional model. Each dimension was further examined by PCA together with physiological data. The final matrix was then scaled by processing the data according to biplot methodology proposed by Gabriel [68]. The resulting multidimensional model represented two contrasted emotional states indicative of high and low arousal of the defence system consistent with anxiety and contentment respectively. The third dimension represented an emotional state
elicited by low arousal of the reward system consistent with frustration. Behaviours included in UQ-ICW, and used in this study, are listed in Table 1.

Application of UQ-ICW in this study

To assess emotional states per day for 10 days, focal sampling of behaviour was done using the videorecord (10 min per hour) for a total of 240 min per day. Rating for day 1 started at hour 2 and was therefore based on 230 min. Video recordings were coded by the experimenter (NG).

Anxious

Per 24 hour period, a cat was scored as Anxious if it engaged in Anxiety behaviour (Table 1) for a minimum of 192 minutes out of 240 minutes of observation. In addition, the cat was never observed sleeping while lying on side, calmly walking around while in an upright position or sitting in an upright posture at the front of the cage.

Frustrated

Per 24 hour period, a cat was scored as Frustrated if it engaged in Frustration behaviour (Table 1) for a minimum of 24 minutes out of 240 minutes of observation. In addition, the cat was never observed engaging in Anxiety behaviours or sleeping while lying on side.

Content

Per 24 hour period, a cat was scored as Content if it engaged in Contentment behaviour (Table 1) for a minimum of 192 minutes out of 240 minutes of observation. In addition, the cat was never observed engaging in Anxiety behaviours or persistent meowing, scanning, repetitive or escape behaviour.

*The experimenter conducting the assessments (NG) was not blind to the study. As the coding of behaviour and rating was done by video following the experiment, the experimenter was familiar with all cats including their grouping (Control or Treated) and cage number.*
Table 1: UQ-ICW behavioural indicators for the rating of Anxious, Frustrated and Content mood

<table>
<thead>
<tr>
<th>Anxiety behaviours observed  &gt; 80 % of observation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flat</td>
</tr>
<tr>
<td>Freeze</td>
</tr>
<tr>
<td>Hide</td>
</tr>
<tr>
<td>Startle</td>
</tr>
<tr>
<td>Crawl</td>
</tr>
<tr>
<td>Retreat</td>
</tr>
<tr>
<td>Self-maintenance</td>
</tr>
<tr>
<td><strong>Absent</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Frustration behaviours observed &gt;10 % of awake time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meow</td>
</tr>
<tr>
<td>Escape bouts</td>
</tr>
<tr>
<td>Scan</td>
</tr>
<tr>
<td>Push</td>
</tr>
<tr>
<td>Pace</td>
</tr>
<tr>
<td>Redirected aggress.</td>
</tr>
<tr>
<td><strong>Bar biting</strong>*</td>
</tr>
<tr>
<td><strong>Absent</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contentment behaviours &gt; 80% of observation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep /rest</td>
</tr>
<tr>
<td>Lie on side</td>
</tr>
<tr>
<td>Front.sit</td>
</tr>
<tr>
<td>Groom</td>
</tr>
<tr>
<td>Walk</td>
</tr>
<tr>
<td>Eat / drink</td>
</tr>
<tr>
<td>Friendly to H</td>
</tr>
<tr>
<td><strong>Absent</strong></td>
</tr>
</tbody>
</table>

*Bar biting was not included in the UQ-ICW (not validated with cortisol or S-IgA) but was added to this study as it was frequently observed together with other Frustration behaviours.*
2.6 Gentling

Gentling was provided for 10 min, 4 times per d, by either the experimenter (NG) or one of two trained volunteers. Treatments started at 0600, 1100, 1600 and 2000 h, with cats being gentled in the same order each time. The exact time that each cat was treated varied slightly depending on the number of cats to be treated each day. All cats to be gentled were first verbally greeted using a high-pitched gentle tone for 30s, with the door closed. The door was then opened with an approach of the experimenter’s hand offered for the cat to sniff.

In this study, Gentling was defined as gentle and short duration repetitive stroking restricted to the head and neck area. Gentling methods were modified to differentially accommodate cats that were anxious, aggressive and friendly at the time.

2.6.1 Gentling: Anxious cats

Cats were initially gentled for 1 min by stroking the cheek, under the chin, and between the ears; with continuous vocal interaction. This was followed by 1 min of withdrawal, during which time the experimenter closed the cage door and stood to the side of the cage out of view, but observing the cat on a computer screen. If the cat stretched his/her neck with attention oriented towards front left of the cage (the location of the experimenter) within 1 min, gentling was initiated immediately. If not, gentling was initiated at the end of the 1 min interruption. This cycle continued for the 10 min period.

2.6.2 Modified Gentling 1: Aggressive cats

If the cat was aggressive during greeting (growling and/or hissing, with or without paw strike), the Gentling was done with the aid of an extendable stick with a rubber tip (Target stick, The Clicker Company, Canada: www.clickercompany.com). The door remained closed; the tool was slid through the bars along the floor and raised up to the cat’s chin initially, then over the cheeks and between the ears. Then the schedule outlined in 2.6.1 was followed. This form of modified gentling was used for 39 sessions out of a total of 2452 (0.015%).

2.6.3 Modified Gentling 2: Friendly cats

If the cat was friendly throughout the greeting (stood, walked, rubbed on experimenter, or walked to the food bowl and ate), gentling was not interrupted.
2.6.4 Control cats

For cats in the Control group, the experimenter stood in front of the cage with the door closed, looking away from the cage and without vocal interaction for 10 min. This was undertaken to ensure that the same level of human presence was experienced by both groups, which therefore only differed in the gentling procedure. This procedure was done after all gentling treatments had been completed.

2.6.5 Rating of response to gentling

The response of cats to the treatment was rated as positive or negative according to behavioural indicators (Table 2), recorded immediately after each treatment.

<table>
<thead>
<tr>
<th>Rating</th>
<th>Description of responses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative</strong></td>
<td>Defensive aggression: onset of hissing, growling with or without paw strike</td>
</tr>
<tr>
<td></td>
<td>Defensive retreat: flattens body and ears, freezes or retreat</td>
</tr>
<tr>
<td><strong>Positive</strong></td>
<td>Calm: relax body, lowers head when petted between ears, raises head when petted on chin. Absence of defensive aggression or retreat</td>
</tr>
<tr>
<td></td>
<td>Friendly: stands close to or walks to experimenter, or remains in sitting or lying down posture, rubs themselves on experimenter’s hands, maintains relaxed body posture; may also walk to food bowl and eat during gentling</td>
</tr>
</tbody>
</table>

2.7 Faeces collection and S-IgA assays

Stools were collected whenever produced, and were weighed and immediately frozen at -40°C. To prepare faecal extracts for IgA ELISA analysis, 10 mL phosphate buffered saline (PBS) containing 10% V/V goat serum (Equitech-Bio, Kerville, Texas, USA) and 0.1 % V/V Kathon (Sigma, Ronkonkoma, New York State, USA) was added to 1g wet faeces and vortexed for 10 min or until fully homogenized. A 2 ml homogenized sample was then centrifuged at 10,000 x g for 10 min at 4°C. Complete, EDTA-free Protease Inhibitor cocktail (Roche Applied Science, Indianapolis, USA) was added to aliquots of the supernatant before storage at -80°C. White ELISA plates (Lumitrac 600, Greiner Bio-one, Frickenhausen, Germany) were coated overnight at 4°C with 100 µL/well goat anti-cat IgA (Bethyl Laboratories Inc., Montgomery, Texas, USA) diluted to 1 µg/mL
in carbonate/bicarbonate coating buffer, pH 9.5. Plates were then blocked for 30 min at 37°C with 200 µL/well coating buffer containing nonfat dry milk (5% W/V) and Kathon (0.1% V/V, Sigma, Ronkonkoma, New York State, USA), then washed three times with phosphate buffer solution containing Tween 20 (0.05% V/V) (PBST). Faecal extracts were diluted in PBST with the addition of nonfat dry milk (5% W/V), normal goat serum (20% V/V), and Kathon (0.1% V/V), starting at 1:1000, and dilutions extended to 1:128,000. Standards (Cat Reference Serum, Bethyl Laboratories Inc., Montgomery, Texas, USA) were diluted to 20 ng/ml, then serially diluted two-fold to 0.31 ng/ml. Diluted samples and standards were added at 100 µL/well in triplicate and incubated at 37°C for 1 h. Plates were washed five times with PBST, then goat anti-cat IgA peroxidase conjugate (Bethyl Laboratories Inc., Montgomery, Texas, USA), diluted 1:100,000 with sample diluent, was added at 100 µL/well and incubated at 37°C for 60 min. Plates were washed five times with PBST and developed with 100 µL/well of SuperSignal ELISA Femto Maximum Sensitivity Substrate (Thermo Scientific, Waltham, Massachusetts, USA) for 2 min. They were read immediately in a multilabel plate reader (Wallac Victor, Perkin Elmer, Waltham, Massachusetts, USA). Prism software (GraphPad Software Inc., California, USA) was used to calculate sample IgA concentrations from a standard curve generated by a four-parameter logistic equation. Concentrations are reported in natural logarithm transformed µg/g of wet faeces. Coefficients of variability were 5.4% and 9.1% for intra and inter assays, respectively. A small number of faecal samples were not analysed due to loss in transport and a spill in the lab.

2.8 Viral and bacterial cultures

Ocular and pharyngeal swabs were performed by a trained veterinary technician immediately upon arrival. Subsequent swabs were obtained on days four and ten for all study cats still at the shelter. Samples were analysed by real-time PCR assays (PCR oligonucleotides and protocols, IDEXX, Westbrook, Maine, USA [36]). Each test used a fluorescent probe that matched with a unique segment of the organism’s DNA or cDNA to ensure high specificity and sensitivity. Real-time PCR was performed with standard primer and probe concentrations (Roche LightCycler® 480 Probes Master mastermix, Roche Applied Science, Indianapolis, USA), default cycling conditions for the Roche LC480 instrument, and a 384-well plate configuration. Cycle thresholds (Ct), or the fractional cycle number at which the fluorescence passes a threshold, were examined to determine if shedding on day four (post vaccination on day 1) represented a true carrier state (street virus) rather than a vaccine-induced shedding. Ct < 29 was considered indicative of abundant target nucleic acid in the sample, representing strong positive reactions, i.e. a heavy pathogen load. Ct of 30-37
indicated moderate amounts of target nucleic acid, and was considered to be a positive reaction (indicative of a moderate pathogen load); and Ct 38-40 indicated minimal amounts of target nucleic acid and was considered a weak reaction (indicative of a minimal viral load). Values > 40 were considered to represent either a very weak infection state or a possible environmental contamination [38].

2.9 Statistical analyses

All tests were assumed to require an alpha of 0.05 for significance. Fisher’s exact test was used to determine if there was a significant difference in cat characteristics at time of enrolment.

2.9.1 The effect of treatment on daily mood and mood change over time.

A Poisson regression analysis was used to compare the daily mood rating for Gentled and Control cats that had been rated as Anxious on arrival. For all Poisson regression analyses, an incidence rate ratio (IRR), confidence interval (CI) and corresponding p-value are reported. The response variables were the number of cats rated as negatively valenced (Anxious or Frustrated), and the number rated as Content each day. The explanatory variables were Gentled/ Control treatment and day. The Poisson model was used in preference to other count models, such as negative binomial or zero-inflated models, because the response variable was not over-dispersed and did not have an excessive number of zeros.

A Student’s t-test was used to determine if the mean length of time to become Content was significantly different between the Gentled and Control groups. A Cox-proportional hazards model was utilized to determine if Gentled cats were more likely to remain content from onset of contentment to the end of the study than Control cats. Hazard ratios (HR), CI and the associated p-values are reported for the model. Generalized estimating equations (GEE) were used to determine if the cat characteristics were significant predictors of daily mood scores, these being appropriate when there are correlations between observations (in this case days for each of the cats).

2.9.2 The influence of mood and response to treatment on S-IgA levels.

GEE were used to determine if daily emotion ratings affected responses to gentling, and if responses varied according to age, source, sex, and sterilization status. A t-test was used to determine if there was a mean difference in the number of stools between treated and control cats. S-IgA values were loge transformed to achieve a normal distribution and a GEE was used to determine if there was a significant difference in S-IgA levels over days. GEE were then used to
determine differences in S-IgA levels between the Gentled and Control groups and between responses (positive, negative) to gentling within the treatment group and according to age, source and gender. Mood ratings on days for which there were no available stools (within 24 hours of rating) were removed from the analysis.

2.9.3 The incidence of shedding and URD by group

Fisher’s exact test was used to determine if the rate of shedding on each of the days, as well as URD levels, were significantly different between Gentled and Control cats. A GEE was used to determine if the level of shedding changed over time. To determine if the time to develop URD was different between the Gentled and Control groups, a t-test was utilized. Fisher’s exact test was used to determine if the incidence of URD was different between treated and control cats (Odds ratio, CI and corresponding p-value are reported). A Cox-Proportional Hazards model was used to compare the incidence of URD between Gentled and Control groups over time. Additionally, GEE were used to determine if S-IgA levels and the incidence of URD differed according to positive or negative response to treatment, and Fisher’s exact test was used to determine if URD differed according to age, source, sex, and sterilization status.

3.0 Results

3.1 Behaviour upon admission

There were no significant differences between Gentled and Control groups for age (seniors, adults, and juveniles), gender (male, female), or source (owner-surrendered, strays) \( (P > 0.05; \text{Table 3}) \).

Table 3: Characteristics of cats in the Gentled and Control groups.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Gentled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Adult</td>
<td>42</td>
<td>60.87</td>
</tr>
<tr>
<td>Juvenile</td>
<td>8</td>
<td>11.59</td>
</tr>
<tr>
<td>Senior</td>
<td>19</td>
<td>27.54</td>
</tr>
<tr>
<td>Intact</td>
<td>22</td>
<td>31.88</td>
</tr>
<tr>
<td>Neutered</td>
<td>47</td>
<td>68.12</td>
</tr>
<tr>
<td>Male</td>
<td>36</td>
<td>52.17</td>
</tr>
<tr>
<td>Female</td>
<td>33</td>
<td>47.83</td>
</tr>
<tr>
<td>Owner-surrendered</td>
<td>41</td>
<td>59.42</td>
</tr>
<tr>
<td>Stray</td>
<td>28</td>
<td>40.58</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>49.6</td>
</tr>
</tbody>
</table>
Of the cats rated as Anxious (n = 139) at intake (day 0), all were negative responders during the Human-Approach Test. However, 81.3% (n = 113) responded with defensive retreat and 18.7% (n = 26) responded with defensive attack. There was no significant difference in assignment to Gentled versus Control groups according to response ($P > 0.05$).

### 3.2 The effect of gentling on daily mood rating

Control cats were more likely than Gentled cats to be rated as Anxious or Frustrated (Incidence Ratio (IRR) $= 0.61$ (0.42-0.88) $P = 0.007$; Fig. 1). Specifically, 276 out of 613 days of observation (45%) were rated as Anxious for Gentled cats versus 333 days (59%) for Controls; and 22 out of 613 days of observation (4%) were rated as Frustrated for Gentled cats versus 30 days (5%) for Controls. Gentled cats were more likely to be rated as Content compared to Control cats (IRR: 1.52, CI: 1.27-1.81, $P < 0.001$, Fig. 2). A total of 315 out of 613 days of observation (51%) were rated as Content for the Gentled group, compared with 202/565 days (35%) for the Control group (GEE $P = 0.02$). Additionally, there was a trend for Gentled cats to be rated as Content sooner: mean days to first rating as Content was $4.25 \pm 2.28$ d for Gentled cats versus $5.07 \pm 2.3$ d for Control cats (t-test $P = 0.09$). Age and gender were not significant predictors of daily mood scores ($P > 0.05$). However, owner-surrendered cats were significantly more likely to be rated as Content compared to stray cats, which in turn were more likely to be rated as Frustrated compared to owner-surrendered cats (Table 4, GEE $P < 0.0001$).

![Fig. 1 Proportion of cats rated as either Anxious or Frustrated over ten days at the shelter in Control and Gentled groups.](image-url)
3.3 The influence of mood and Gentling treatment on S-IgA levels

Within the Gentled group, there was a significant difference in negative versus positive responders (Table 5) according to the cats’ mood rating on that day. Cats were significantly more likely to respond positively to gentling on days when they were rated as Content (86% positive responses) compared to days when they were rated as Anxious or Frustrated (68% and 27% respectively, $P$
Chapter 3

Gender, age and source were not significant predictors of response to treatment ($P>0.05$).

Table 5: Positive and negative responses of cats in the Gentling treatment according to their daily mood classification of Content, Anxious or Frustrated.

<table>
<thead>
<tr>
<th>Mood Rating (n= days)</th>
<th>Negative</th>
<th>Positive</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content (315 days)</td>
<td>43</td>
<td>272</td>
<td>0.0001</td>
</tr>
<tr>
<td>Anxious (226 days)</td>
<td>73</td>
<td>153</td>
<td>0.0001</td>
</tr>
<tr>
<td>Frustrated (22 days)</td>
<td>16</td>
<td>6</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

There was no significant difference in the mean number of stools analysed between the groups (Control =3.8 ±1.6, Gentled =4.1 ±1.7, $P=0.24$). S-IgA was higher in the Gentled (6.9 ±0.7 log$_e$ µg/g) than the Control cats (5.9 ±0.5 µg/g) ($P<0.001$), and a significant increase over days was found in both groups (GEE, $P<0.0001$) (Fig.3).

![Fig. 3 Generalized estimated equations comparing day effect on S-IgA log$_e$ µg/g for Gentled and Control groups.](image)

When considering all cats that were rated Anxious on arrival, S-IgA values were greater for cats rated as Content (7.01 ±0.7 log$_e$ µg/g) than those rated as Anxious (6.61±0.7 log$_e$ µg/g) during the trial, which were in turn greater than for cats rated as Frustrated (5.9 ±0.4 log$_e$ µg/g) ($P<0.0001$). S-IgA values were also greater for Gentled (6.62 ±0.7 log$_e$ µg/g) than Control cats (5.75 ±0.8 log$_e$ µg/g) ($P<0.0001$), even if they were rated as Content (Gentled 7.01 ±0.7; Control 6.32±0.7 µg/g; $P$ <0.0001).
Furthermore, S-IgA was significantly greater for positive ($7.03 \pm 0.6 \log_e \mu g/g$) than negative responders ($6.14 \pm 0.8 \log_e \mu g/g$) to gentling ($P = 0.001$; Table 6). There was no significant effect of source, age, gender or sterilization status on S-IgA $\mu g/g$ ($P > 0.05$).

Table 6: Mean S-IgA concentrations in faeces for Gentled cats that responded either positively and negatively to gentling.

<table>
<thead>
<tr>
<th>Groups</th>
<th>S-IgA Log$_e$ $\mu g/g$</th>
<th>SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.75</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Gentled</td>
<td>6.62</td>
<td>0.7</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Negative response</td>
<td>6.14</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Positive response</td>
<td>7.03</td>
<td>0.6</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

3.4 The effect of gentling on incidence of viral and bacterial shedding and URD by group

The Gentling and Control groups did not differ for shedding rate of all pathogens upon admission (Fisher’s exact test $P > 0.05$). There was a significant increase in shedding over time in non-gentled cats (23%, 35%, 52% on days 1, 4 and 10, respectively), but not gentled cats (32%, 26%, 30% on days 1, 4 and 10, respectively) ($P = 0.001$).

Control cats were 2.37 (HR; CI 1.35-4.15) times more likely to develop URD over time as cats that received gentling treatment ($P < 0.0001$; Fig.4). The onset of clinical URD occurred significantly earlier for Control (mean 8.8 ±11.76 d) than Gentled cats (mean 18.5 ±5.63 d) ($P = 0.001$). Within both groups, the incidence of URD was greatest in cats rated as Frustrated (50%), compared with cats rated as Content (28%) or Anxious (36%) ($P < 0.0001$). Of the 69 cats in the control group, 31 cats (44.9%) developed URD. Of the 70 cats in the treatment group, 22 cats (31.4%) developed URD. Thus there was a reduction of 13.5% in URD in cats receiving treatments. Source, age, gender and response to gentling were not predictors of the incidence of URD ($P > 0.05$).
4.0 Discussion

Alleviating emotional pain is of clinical importance pursuant to the Veterinarian Oath [5]. Positive interactions with humans are a valued activity for cats in homes, whether they are allowed access to the outside or kept strictly indoors [12]. The current findings indicate that positive human interaction, in the form of gentling, can enhance emotional wellbeing, enhance mucosal immunity and decrease the incidence of URD in shelter cats.

Gentled cats were rated as Content sooner and more frequently than non-gentled cats. Similar effects have been observed in dogs. Decreased heart rate and cortisol is more pronounced in dogs that display friendly behaviour towards the handler [14]. Although these data confirmed previous findings [39] that Content cats produce more S-IgA than Anxious cats, in the current study, Content cats that were gentled showed a significantly higher concentration of S-IgA compared to those that were not gentled. Similar results have been observed in humans. In an experiment where anxiety was measured before and after a 10 min back rub versus 10 min of quiet relaxation on the massage table, both groups showed a similar decrease in anxiety. However, salivary IgA only increased in the group receiving the back rub [40]. The specific effect of gentling on S-IgA, in addition to its positive effect on emotions, is of unknown aetiology. Gentling may have induced changes in physiology that may have contributed to enhancement of mucosal immunity. In rats [41] and dogs [42], gentle petting increases oxytocin. This neurochemical is known to have many benefits on wellbeing [43-45]. In addition, it was found that in shelter cats, positive
interactions with one person seem to increase positive responses to unfamiliar people [46]. In rats, gentling reduces fear during subsequent exposure to a fear provoking open-field test [47]. Thus, gentling may have increased the benefits of exposure to people, even during the cleaning procedure by staff.

The scale used in this study (UQ-ICW) was shown to accurately identify negative and positive affect as evidenced by immunological correlates. However, variation in emotional arousal cannot be determined by this scale in its current form. According to the differences found in Content gentled versus non-gentled cats, it may be useful to determine immunological correlates of positive affect according to the level of emotional arousal. Further research may be necessary to refine this scale. Moreover, in this study three types of gentling were used, but not specifically compared to each other in terms of benefits to the wellbeing of cats. Therefore, it could not be determined which aspect of gentling was most beneficial to the cats.

Cats too aggressive to handle by hand were provided with mechanical gentling using an extendable stick equipped with a rubber tip. This technique produced a rapid decrease in anxiety, which in turn was associated with an increase in S-IgA production. In animals with a tendency to fear humans, such as ewes [48, 49] and cattle [50], mechanical restraint has a calming effect, compared to being handled by a human. These findings have important implications for the welfare of fearful cats in institutional settings. In North American shelters (and probably worldwide), staff are called upon to determine if cats showing fear may be feral, because they cannot be socialized past the age of 3 months [51], or if they may be socialized, but fearful non-feral cats. This is a difficult task, and cats classified as feral are routinely euthanized following a legal holding period of (usually) 3 or 4 days [52]. According to our observations, 18% of the cats in this study would have been candidates for euthanasia within that holding period, based on their aggressive response to the Human-Approach Test and to gentling. However, our research protocol required all cats to be kept for 10 days prior to staff making an adoption/euthanasia decision, during which time aggressive cats received mechanical gentling if they could not be safely handled. Among the gentled cats, none responded with aggression after day six. Thus, a 3 to 4 day holding period may not be sufficient to differentiate non-feral from feral cats.

Our data confirms that emotional stress can induce viral reactivation in cats with subclinical infections [53]. We observed a significant increase in shedding by day four in non-gentled, but not gentled cats. Pedersen et al. [54] proposed that the onset of shedding within a few days at the shelter may be due the reactivation of a latent infection rather than infection contracted on-site. Non-gentled cats also showed significantly higher incidence of URD, with onset of clinical signs occurring sooner than for gentled cats that became sick. In both cases, our conclusions are in
accordance with many researchers who propose that stress management should be part of disease management practices in shelters [8, 55, 56]. However, the importance of qualifying the source of stress was also evident in our findings.

The incidence of URD was greater for cats that were categorized as Frustrated compared to Anxious cats. It has been proposed that for humans [57] and veterinary species [8], interventions should address any specific emotional problem that may be affecting health. Gentling can reduce fear and anxiety by reducing arousal of the emotional defence system; however, it does not address the underlying cause of frustration such as behavioural restriction, non-reward or unpredictable appetitive events [58-61]. Frustration is rarely reported in shelter cats, although persistent meowing [62] and pacing [63] have been observed. Our findings show that frustration is a significant risk factor for URD indicating the need for further research on identification and remediation of frustration in shelter cats.

5.0 Conclusion

Gentling induced positive affect (contentment) and increased secretory immunoglobulin values in faeces. Gentled cats were significantly less likely to develop clinical signs of URD over time than Control cats.

References

Chapter 4: Effect of cognitive enrichment on behaviour, secretory immunoglobulin A and incidence of respiratory disease in frustrated shelter cats.

Abstract

Acquisition of resources and opportunity to engage in natural behaviours was shown to reduce frustration-related behaviours and enhance immunity and health in some farm animal species but little is known about similar effect in domestic cats living in confinement in animal shelters. Fifteen cats assessed as frustrated on entry to a shelter were assigned to either a Treatment (n=7) or Control (n=8) group. Treatment cats were given access to a 4 m² room four times daily for 10 min over a 10 d period and took part training sessions during which time they could acquire a food reward in response to appropriate responses to learning task. A daily mood rating (Frustrated, Apathetic or Content) was assigned to each cat according to emotional classification of the University of Queensland Indices of Cat Wellbeing (UQ-ICW). Treated cats were 1.9 times more likely to be rated as Content than the Control cats (CI: 1.01-3.68, P <0.0001). Total feline faecal IgA was quantified by enzyme-linked immunosorbent assay, and treated cats had greater concentrations of S-IgA (6.7±0.5 log e µg/g) than Control cats (6.1±0.6 µg/g, P=0.03). Within the Treatment group, cats rated as Content had greater concentrations of S-IgA (6.8±0.4 log e µg/g) than those that remained Frustrated (6.0±0.1 log e µg/g) (P <0.001). Incidence of clinical upper respiratory disease, URD, was determined based on onset of ocular or nasal discharge. Control cats were more likely to develop URD overtime compared to cats that received treatment (HR: 2.37, CI: 1.35-4.15, P <0.0001). It is concluded that cognitive enrichment of cats exhibiting behaviours suggesting frustration can elicit positive affect (contentment), stimulate secretion of IgA and reduced incidence of upper respiratory disease.

Keywords: Clicker training, Frustration, Respiratory disease; Secretory Immunoglobulin A; Shelter cats.
1.0 Introduction

Due to their strong motivation to hunt, forage and range, carnivores may be particularly susceptible to frustration when confined [1]. Although domestic cats are believed to adapt well to confinement [2], there is also evidence that it can affect their wellbeing. Thwarted access to the outdoors may aggravate cystitis [3-6] contribute to the onset of type 2 diabetes mellitus [7], and aggravate behaviour problems such psychogenic dermatitis (skin irritation due to over grooming), pica [8], inter-cat aggression [9] and may cause redirected aggression towards a human [10]. Various conditions associated with shelter life are likely to cause frustration.

For household cats, feeding is usually predictable in terms of time and location [11]. In laboratory cats, unpredictable husbandry (such as delayed feeding) increases adrenocortical output [12]. As the feeding schedule may vary daily in an animal shelter due to the large number of cats to be fed, shelter cats may experience similar effects. It has been proposed that uncontrollable and unpredictable appetitive events are as stressful as uncontrollable and unpredictable aversive events [13].

Early experiments indicated that stimulation of the tegmentum (in the midbrain) could be used as a reinforcer for food consumption in aphagic (refusal to eat dry food) [14]. Subsequent investigations established that such brain stimulation activates dopamine neurons which serve to reinforce responses to rewards [15]. Dopamine neurons fire a burst of spikes when the reward value is greater than expected conversely they are inhibited when it is of lesser value than expected [16]. Hence dopamine has been associated with the experience of pleasure [17]. Conversely, rewards with lesser value than expected, delay or inability to acquire desired resources affects dopamine transmission associated with symptoms such as frustration, anhedonia (inability to experience pleasure) and apathy [18-20]. Under confinement, the inability to engage in behaviours normally associated with acquisition of resources (roaming, foraging, hunting) may elicit performance of behaviours in a repetitive or stereotypic fashion (such as pacing) or seemingly unrelated behaviours (such as self-mutilation) [21-23]. It has been proposed that spending more than 10% of waking time engaged in stereotypic behaviour is an indicator that an animal is frustrated [24]. Loss of enrichment is another important source of frustration. In mice, removal of enrichment can elicit stereotypic behaviours [25]. For the most part cats, entering an animal shelter experience a loss of enrichment such as sensory access to the external environment in addition to behavioural restriction and restricted access to valued resources (such as human companionship) [26] all of which may cause frustration. In comparison fear and anxiety related behaviour, those that may be induced by frustration have received less attention. Pacing, escape attempts [27], and persistent meowing [28, 29] have been
observed in caged cats and may be indicative of frustration. Chronic frustration may also be a risk factor for a loss of motivation to engage in any behaviour, including eating. Apathy has been observed following repeated failures to perform natural behaviours or obtain a desired resource [30]. Frustration has been linked to aggression [31] in many species, including humans. In cats confined to the home, the sighting of another cat through a window can cause the animal to attack any available target [10]. In 95% of Canadian animal shelters, aggression is a criterion for euthanasia [32].

Distinguishing frustration from other emotional states experienced by cats in shelters may also have important health implications. Electro stimulation of dorsal, ventral and lateral hypothalamus in cats induces defensive attack (associated with fear), defensive retreat (associated with anxiety) and restlessness (frustration), respectively [33]. The authors reported an increase in plasma cortisol, indicating stress in the three states, but only restlessness and defensive attack were associated with an increase in a lymphocyte proliferation in response to phytohemagglutinin while defensive retreat was not. Thus, various emotional states are differentially correlated with cell-mediated immunity in cats. Differentiating frustration from other negative states, such as anxiety or fear, may facilitate the development of appropriate interventions. For example, provisions for hiding that are beneficial for fearful shelter cats may not be of value to cats motivated by a desire to exit the cage. The benefits of interventions for zoo animals experiencing frustration are widely discussed, but remain unexplored for shelter cats. However, for cats experiencing frustration in response to confinement to the home, increased access to toys or engaging the cat in mentally stimulating activities by teaching a novel behaviour has been recommended [34]. Learning novel behaviours with the use of a clicker device (employing a click sound as a secondary reinforcer) is promoted by the Humane Society of the United States [35] and many courses designed for the shelter environment are available [36]. However, scientific examination of the welfare benefits of clicker training for cats is sparse. One study [37] reported that cats tended to show quieter postures while waiting for a reward during clicker training sessions. Quiet behaviour as an anticipatory response may reflect the natural food-search behaviour typical of cats [38].

In this study, we tested the hypothesis that frustrated cats may benefit from a period out of the cage to participate in learning a task that gave them control over the delivery of consummatory rewards. Towards that end, we examined changes in frustration over time, and associated levels of S-IgA, in response to a training session with food reward away from the home cage. We also examined the effect of treatment on viral shedding and incidence of URD.
2.0 Method

This study was approved by the University of Queensland Animal Ethics Committee (CAWE/231/10).

This study was part of a larger research project designed to examine the effects of behavioural interventions on cats rated as anxious, frustrated or content upon admission (see Chapter 2 for detailed methods). In brief, the study took place at the Vancouver Branch of the British Columbia Society for the Prevention of Cruelty to Animal (BC SPCA, Vancouver, Canada). Cats were either surrendered by their owner or brought in as strays by a humane officer between May and November 2010. Age was either provided by the owners or estimated by shelter staff, and categorized as juvenile (6-12 months), adult (1-7 years), or senior (>8 years). Cats were examined upon admission by an animal health technician, vaccinated (Fel-O-Guard+3 Boehringer Ingelheim Ltd., Burlington, Ontario, Canada), and dewormed (Strongid® T, Pfizer, Quebec, Canada). Combined conjunctival and oropharyngeal swab specimens were collected before vaccination during the initial examination and on days 4 and 10. Samples were tested by quantitative real-time PCR [39] for feline herpesvirus type 1 (FHV-1), feline calicivirus (FCV), Mycoplasma felis, Chlamydophila felis, and Bordetella bronchiseptica. Stools were collected daily, as available, and total feline faecal IgA was quantified by enzyme-linked immunosorbent assay (ELISA). Following examination, cats were transported in a small wire cage (covered with a towel) to their cage (76 x 76 x 71 cm), which was furnished with litter boxes, food and water bowls and towels for bedding, in a study ward. Feed was provided twice daily at 0700 and 1700 h) (Science Diet, Hill's Pet Nutrition, Inc. ®/™, Vancouver, Canada) and fresh water was provided ad libitum. Cages were cleaned daily by removing all waste, changing bedding and wiping walls with a clean cloth soaked in water; and cages were disinfected between cats with a 1% disinfectant solution (Virkon®, Du Pont, Mississauga, Ontario, Canada) and water (Antec International Limited, Sudbury, UK).

2.1 Behavioural observation upon admission (day 0)

A 1 h real-time video observation commenced as soon as the cat was placed in the cage. This was followed by a Human-Approach Test, adapted from Kessler and Turner [40]: Step 1: the experimenter stood in front of the cage without interaction such as eye contact or verbal greeting (2 min); Step 2: the experimenter talked to the cat using a high pitched, gentle tone and had some eye contact, but with eyes half closed) (1 min); Step 3: the same procedure was repeated with the door open, followed by an approach of the hand so that it was near the cat (2 min). If the cat attempted to attack (bite or paw strike with hiss and/or growl) the door was closed immediately.
Cats were rated as Frustrated if they engaged in one or more of the following behaviours: persistent meowing, scanning of the cage, pushing on the door, pawing the walls or floor, pushing their paw through the bars and if they attempted to escape during the Human-Approach Test. In addition, the Frustrated rating was only given if there was an absence of hiding, flat posture and defensive retreat.

2.2 Rating of emotional states each day (mood)

Mood is defined as an emotional response lasting more than a few hours [41]. We measured changes in mood over time based on focal sampling of behaviour from the videorecord (10 min per h over 10 days). Rating of daily mood was done according to behavioural indicators of Frustration and Contentment identified in a previous study that produced the University of Queensland Indices of Cat Wellbeing (UQ-ICW) (Chapter 2). The rating of Anxious was removed from the ethogram as no cats were observed engaging in Anxiety behaviours in this study. However, UQ-ICW was adapted to accommodate for behaviours observed in this study such as bar biting, hypersomnia, lack of interest in surroundings and people and absence of eating and grooming behaviour (collectively termed apathy).

Application of UQ-ICW in this study

To assess emotional states per day for 10 days, focal sampling of behaviour was done using the videorecord (10 min per hour) for a total of 240 min per day. Rating for day 1 started at hour 2 and was therefore based on 230 min. Video recordings were coded by the experimenter (NG).

Frustrated

Per 24 hour period, a cat was scored as Frustrated if it engaged in Frustration behaviour (Table 1) for a minimum of 24 minutes out of 240 minutes of observation. In addition, the cat was never observed engaging in Anxiety behaviours or sleeping while lying on side.

Content

Per 24 hour period, a cat was scored as Content if it engaged in Contentment behaviour (Table 1) for a minimum of 192 minutes out of 240 minutes of observation. In addition, the cat was never observed engaging in Anxiety behaviours or persistent meowing, scanning, repetitive or escape behaviour.
Apathetic

Per 24 hour period, a cat was scored as apathetic if it observed sleeping for at least 192 minutes out of the 240 minutes of observation and was not observed eating, grooming or interacting with humans with hypersomnia.

*The experimenter conducting the assessments (NG) was not blind to the study. As the coding of behaviour and rating was done by video following the experiment, the experimenter was familiar with all cats including their grouping (Control or Treated) and cage number.*
Table 1: UQ-ICW behavioural indicators used for the rating of Frustrated and Content mood.

<table>
<thead>
<tr>
<th></th>
<th>Frustration behaviours &gt; 10% of awake time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meow</td>
<td>Persistent meow not related to anticipation of food)</td>
</tr>
<tr>
<td>Escape bouts</td>
<td>Very active and includes one or more behaviours performed persistently or frequent in repeated bouts and occurs mainly at the front of the cage: On hind limbs, pawing wall/ floor, paw through door, push on door or latch, hanging on cage door with body inverted</td>
</tr>
<tr>
<td>Scan</td>
<td>Visual scanning of the cage</td>
</tr>
<tr>
<td>Push</td>
<td>Hits or throws objects around the cage in a destructive manner using head, body or paws (not related to play.) Spills food bowls, and litter. May push on cage door with head, paws or body.</td>
</tr>
<tr>
<td>Pace</td>
<td>Repetitive and fast walking back and forth in the cage particularly by the door</td>
</tr>
<tr>
<td>Redirected aggress.</td>
<td>Quick bite, swipe or scratch during interaction with human without defensive vocalizations, body posture or retreat. May occur in bouts during otherwise friendly interactions</td>
</tr>
<tr>
<td>Bar biting*</td>
<td>Bites or lick cage bars persistently or in repeated bouts</td>
</tr>
<tr>
<td>Absent</td>
<td>All Anxious behaviours and sleeping while lying on side</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Contentment behaviours &gt; 80% of observation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep /rest</td>
<td>Relax body posture and neck, eyes closed or semi closed</td>
</tr>
<tr>
<td>Lie on side</td>
<td>Laterally recumbent, body stretched, neck and ventral area exposed, limbs and tail extended</td>
</tr>
<tr>
<td>Front.sit</td>
<td>Sits at the front of the cage, calmly looking around</td>
</tr>
<tr>
<td>Groom</td>
<td>Licks fur or paws and rubs head with paws (without chewing or pulling coat)</td>
</tr>
<tr>
<td>Walk</td>
<td>High body posture, normal gait and pace to move around the cage (not on-going or repetitive)</td>
</tr>
<tr>
<td>Eat / drink</td>
<td>Takes food or water into mouth and ingest. Particularly in the presence of humans. Does spill food or water around the cage</td>
</tr>
<tr>
<td>Friendly to H</td>
<td>Approaches front of cage or human in amicable manner.</td>
</tr>
<tr>
<td>Absent</td>
<td>All Anxious behaviour, persistent meowing, scanning, repetitive or escape behaviour</td>
</tr>
</tbody>
</table>

*Bar biting was not included in the UQ-ICW (not validated with cortisol or S-IgA) but was added to this study as it was frequently observed together with other Frustration behaviours.

Apathetic could not be included as an emotion as it was not validated during the construction of the UQ-ICW. However this classification was assigned to cats in this study based on observations indicating a lack of motivation [42].

Indicators were: Absence of feeding (unless human intervention was provided by medical staff such as placing food in the cats mouth using a tongue depressor), grooming and interaction with humans together with hypersomnia.
2.3 Treatment procedure

Interventions were provided four times per day by one experimenter (NG), thus not all cats could receive treatment at the same time. Treatments started at 6:00, 11:00, 16:00 and 20:00h. Sessions took place in a 4m² treatment room adjacent to the housing unit. Where necessary, cats were held under the experimenter’s arm and carried to the room; after a few sessions most cats jumped out of the cage and walked to the treatment room unassisted. Sessions started with 2 min of free exploration. Shaping of the desired behaviour (high five: contact between the cat’s paw and the experimenter’s hand) was initially started by conditioning the cat to the clicker sound (The Clicker Company, Payson, Arizona, USA) for about 1 min (click sound followed by delivery of food reward). The behavioural sequence for high five was sit, raise paw to shoulder height, touch handler’s hand (and sustain contact for a few seconds). Shaping was done by first rewarding the sit behaviour if it occurred naturally or if lured into position using a target stick. Luring cats into a sit was achieved by holding the stick near the cat’s nose and rewarding nose to stick contact and subsequently moving it backwards until the cat was in a sit position. Any movement of the right or left paw was rewarded. When the paw lift reached the cat’s shoulder height, the handler placed her hand in the path of the movement and rewarded contact. Reward was delayed very briefly to extend contact time of the cat’s paw with the handler’s hand. Once the sequence was learned, the behaviour was requested verbally “give me five” and by placing the hand near the cat. At the end the training session, the cat was picked up and placed back in the cage.

2.4 Rating of response to treatment

Response to treatment was noted immediately after each session. It was rated as negative if they spent their time pacing at the door or window, if they avoided, growled or hissed when approached by the experimenter. It was rated as positive if they interacted with the experimenter in a friendly manner (whether they learned the task or not), remained in close proximity and expressed behaviours such as tail up, purring, rubbing, head butting, chirping or meowing or attempted to climb on the handlers lap.

2.5 Statistical analysis

All tests were assumed to require an alpha of 0.05 for significance. Fisher’s exact test was used to determine if there was a significant difference in cat characteristics at the time of enrolment. To determine if there was a significant difference in the proportion of cats that had a particular daily mood, a Poisson regression analysis was used to compare Treated and Control cats that had been rated Frustrated on arrival. For all Poisson regression analyses an incidence rate ratio (IRR),
confidence interval (CI) and corresponding p-value are reported. The response variable was the number of cats rated as Content or Frustrated each day and the explanatory variables were Treated/Control treatment and day. The poisson model was used in preference to other count models, such as the negative binomial or zero-inflated models, because the response variable was not over-dispersed and did not have an excessive number of zeros.

A Student’s t-test was used to determine if the mean length of time to becoming Content was significantly different between the Treated and Control groups. A Cox-proportional hazards model was utilized to determine if Treated cats were more likely to remain content from the onset of contentment to end of the study than Control cats, hazard ratios (HR); confidence intervals and the associated p-value are reported. Generalized estimating equations (GEE) were used to determine if the cat characteristics were significant predictors of daily mood scores. GEE are used when there are correlations between observations (in this case days for each of the cats) to determine if there are associations with the factors.

GEE were used to determine if the mood of cats that responded to treatment was significantly different than that of cats that did not respond to treatment. Furthermore, GEE were used to determine if response to treatment varied according to age, source and gender. A t-test was used to determine if there was a mean difference in the number of stools between treated and control cats. S-IgA values were loge transformed to achieve a normal distribution. A GEE was used to determine if there was a significant difference in S-IgA levels depending upon the day. The GEE was then used to determine differences in S-IgA levels between the Treated and Control groups and between responses (positive or negative) to training within the group and according to age, source and gender. Mood ratings on days for which there were no available stools (within 24 hours of rating) were removed from the analysis.

Fisher’s exact test was used to determine if the rate of shedding on each of the days, as well as URD levels, were significantly different between Treated and Control groups. A Cox-Proportional Hazards model was used to compare the incidence of URD between Treated and Control groups over time. Fisher’s exact test was used to determine if incidence of URD differed according to response to treatment (positive or negative) and according to age, source and sex.
3.0 Results

There was no significant difference in the distribution of cats as a result of their entry characteristics or treatment (Fisher’s Exact test \( P >0.05 \)) (Table 2).

3.1 Behavioural observation on admission (day 0)

Of the 250 cats assessed upon admission, 15 (6%) were rated as Frustrated. None of the cats responded negatively (aggression, withdrawal or attempt to escape) during the Human-Approach Test.

Table 2: Characteristics of cats in the Treatment and Control groups.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>total</td>
<td>N 8</td>
<td>N 7</td>
</tr>
<tr>
<td>Adult</td>
<td>4 50.0</td>
<td>4 57.1</td>
</tr>
<tr>
<td>Juvenile</td>
<td>3 37.5</td>
<td>3 42.9</td>
</tr>
<tr>
<td>Senior</td>
<td>1 12.5</td>
<td>0 0.0</td>
</tr>
<tr>
<td>Intact</td>
<td>8 100.0</td>
<td>7 100.0</td>
</tr>
<tr>
<td>Neutered</td>
<td>0 0.0</td>
<td>0 0.0</td>
</tr>
<tr>
<td>Male</td>
<td>3 37.5</td>
<td>5 71.4</td>
</tr>
<tr>
<td>Female</td>
<td>5 62.5</td>
<td>2 28.6</td>
</tr>
<tr>
<td>Owner-surrendered</td>
<td>2 25.0</td>
<td>2 28.6</td>
</tr>
<tr>
<td>Stray</td>
<td>6 75.0</td>
<td>5 71.4</td>
</tr>
</tbody>
</table>

3.2 Daily mood assessment and the effect of mood on response to treatment

There was a trend for Treatment cats to have a lower incidence of frustration than Control cats (IRR: 0.66, CI: 0.43-1.01, \( P =0.058 \), Fig.1). Treatment cats were 1.93 times more likely to be rated as Content than Control cats (IRR: 1.93, CI: 1.01-3.68, \( P <0.0001 \), Fig.2). As summarized in Table 3, out of 67 days of observation for the Control cats, 49 days (73 %) were rated as negatively valenced: 41 (61.2%) days as Frustrated and 8 (11.9%) as Apathetic (mean days to apathy = 6). Within the Treatment group, out of 55 days of observation, 28 days (51%) were rated as negatively valenced (51% and 0% Frustrated and Apathetic, respectively). Additionally, there was a trend for Treatment cats to be rated as Content sooner. Mean days to first rating as Content for Treatment cats was 3.2 (±1.3 days) versus 4.4 days (±0.3) for Control cats (t-test \( P =0.09 \)). Once the Treated cats became Content they tended to remain Content until the end of the study, compared to Control.
cats (HR: 3.64, CI: 0.85-15.67, $P = 0.08$). Mood rating was not affected by sex, age, sterilization status or source ($P > 0.5$).

Table 3: Proportion of cats per rating category and days to first rating for Treated and Control groups. All cats were rated as Frustrated upon admission.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Frustrated</th>
<th>Content</th>
<th>Apathetic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (67)</td>
<td>41 (61.2%)</td>
<td>18 (26.8%)</td>
<td>8 (11.9%)</td>
<td></td>
</tr>
<tr>
<td>Treated (55)</td>
<td>28 (51%)</td>
<td>27 (49%)</td>
<td>0 (0%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Days to onset</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Day1</td>
<td>4.4 ± 0.5 (5 cats)</td>
<td>6 ± 0 (2 cats)</td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>Day1</td>
<td>3.2 ± 1.3 (4 cats)</td>
<td>0 cats</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Fig. 1: Proportion of cats rated as Frustrated over the 10 days in Control and Treatment groups
3.3 Influence of mood and response to treatment on S-IgA levels

Proportionately, more cats had a positive response to treatment on days when they were rated as Content, compared to days when they were rated as Frustrated ($P < 0.0001$) (Table 4). Males and owner-surrendered cats were significantly more likely to respond positively to treatment than females and stray cats ($P = 0.04$). Age did not affect response to treatment ($P = 0.17$).

Table 4: Positive and negative responses to treatment according to Mood rating of Frustrated or Content.

<table>
<thead>
<tr>
<th>Mood Rating (n= days)</th>
<th>Negative</th>
<th>Positive</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content (27 days)</td>
<td>2</td>
<td>25</td>
<td>0.07</td>
</tr>
<tr>
<td>Frustrated (28 days)</td>
<td>5</td>
<td>23</td>
<td>0.18</td>
</tr>
</tbody>
</table>

There was no significant difference in the mean number of stools analysed between the groups (control = $3.6 \pm 1.5$/cat, treated = $4.6 \pm 2.7$/cat, $P = 0.24$). There was no significant effect of day on S-IgA for either group (Controls: $P = 0.88$) (Treated: $P = 0.30$). S-IgA values were greater for Treatment ($6.73 \pm 0.47 \log_e \mu g/g$) than for Control cats ($6.04 \pm 0.68 \log_e \mu g/g$) ($P = 0.03$), and for Treated cats that responded to treatment positively ($6.83 \pm 0.41 \log_e \mu g/g$) versus negatively ($5.99 \pm 0.14 \log_e \mu g/g$) (GEE $P = 0.003$) (Table 5). There was a tendency for S-IgA to be greater in Treated cats rated as Content ($6.83 \pm 0.41 \log_e \mu g/g$), compared with Control cats rated as Content.
(6.56 ±0.23 loge µg/g) (GEE \( P =0.06 \)). There was no significant difference in S-IgA according to source, age or sex (\( P >0.05 \)).

Table 5: Mean S-IgA values (loge µg/g) by group and according to response to treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.04</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>6.73</td>
<td>0.47</td>
<td>0.027</td>
</tr>
<tr>
<td>Positive response</td>
<td>6.83</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Negative response</td>
<td>5.99</td>
<td>0.4</td>
<td>0.003</td>
</tr>
</tbody>
</table>

3.4 Effect of treatment on viral shedding and incidence of clinical URD

There were no differences in shedding rate (for all pathogens) at admission (42.9 % versus 25.0 %) or on days 4 (50.0 % versus 37.5 %) and 10 (57.1 % versus 60.0 %) between Treatment (n=7) and Control (n=8) cats (Fisher’s exact test \( P >0.05 \)). Only one Treatment cat (14%) developed URD, on day 23, compared with four (50%) of the Control cats (mean = d 7 ±2.9). The Cox-Proportional Hazards model demonstrated that Control cats were more likely (HR: 2.37, CI: 1.35-4.15) to develop URD overtime compared to Treatment cats (\( P <0.0001 \)). Response to treatment was not a predictor of incidence of URD (\( P >0.05 \)). There was no difference in incidence of clinical URD arising from source, age or sex (\( P >0.05 \)).

4.0 Discussion

Although many characteristics of the domestic cat have been modified over 4000 years of domestication [43], there is also evidence that many behavioural characteristics of a species are not changed by domestication [44]. In the case of cats, they can return to a feral state in one generation. Kittens born outside, without human contact until the age of 12 weeks, cannot be re-socialized and remain fearful of humans throughout their lifespan [45]. Even when well socialized and fed by humans, they retain many ethological characteristics of their wild counterparts, such as roaming and hunting [46]. For many carnivore species, confinement induces persistent and enduring stereotypic behaviour due to their strong drive to hunt, roam, forage, and range; and their high levels of stamina [1]. In wild species (serval cats and tigers), enrichment that fulfils natural drives, such as the provision of artificial prey, can reduce pacing [47]. However, accounts of frustration in caged domestic cats are sparse. Ottway [48] reported pacing in only one
cat and McCune [27] reported persistent meowing and pacing in cats as little as day 2 post-admission. In this study of 250 cats observed during their first hour of confinement, only 15 showed behavioural indicators of frustration. However, this does not necessarily suggest that domestic cats are not frustrated by confinement. In our previous studies (Chapter 3), many cats experienced the onset of frustration later on during their stay [49]. In addition, frustration-related behaviours, with the exception of pacing and persistent meowing, occur in short spurts (>10% of waking hours) during which the cat is pushing items around the cage, biting bars, pushing on the latch, hanging upside down on the bar and generally destroying items in the cage. Thus, depending on the sampling schedule, these behaviours can easily be missed. In this study, in addition to observing the behaviours, we also used environmental cues such as a disrupted cage in which the contents within it are repositioned and spilt. A disrupted cage can also be an indicator of anxiety, however. Anxiously moving items around the cage to create a hiding area [27], mostly occurs at night and mostly in cats which are immobile during the rest of the day. Because of that, we also used environmental cues such as obvious destruction of cage items (which were also observed with behaviours such as pushing items around not related to play).

A behaviour observed in Frustrated cats (paws through door) is commonly interpreted as a cat being social and used by adopters as a criterion for selection of an individual for adoption [50]. In this study it was believed to be an indicator of Frustration due to the correlation to behaviours such as bar biting, hanging upside down on the cage railing and persistent attempts to open the cage latch.

The effect of treatment for Frustration indicated that it was effective at inducing a state of contentment. Although Frustration decreased similarly in all cats over the 10 days of observation, treated cats were subsequently rated as Content whereas control cats tended towards apathy (hypersomnia with little interest in feeding, grooming, and social interactions with humans). Apathy and particularly loss of interest in feeding may be indicative of sickness [51], but can also be an indicator of also occurs in animals that experience repeated failure to escape confinement, failed attempts to avoid aversive conditions, or under conditions of social isolation [23, 24, 52]. Sleeping is commonly used as an indicator of good welfare in shelter cats [29]. However, we agree with McCobb [53] that it can also be an indication of depression in this species and should be determined in terms of amount and correlation to other indicators. Gouveia and colleagues [54] evaluated long term shelter residents, finding that cats became more inactive the longer they spent at the shelter.
We had expected to find that males and strays experience more sustained frustration, as males tend to roam more than females when living outdoors [55] and make use of more space within the home [56]; and strays are likely to have had a lifestyle with more freedom than cats confined to the home. However, there was no significant difference in Frustration, Contentment, or Apathy by sex, source, or neuter status. Dydall and colleagues [57] reported lower behavioural stress scores in strays compared to cats surrendered by their owners. However, their findings also show that twice as many strays were subsequently euthanized for disease (most often URD) than owner-surrendered cats. However, no difference by source has also been reported [53].

The positive effect of our treatment was supported by greater levels of S-IgA in cats receiving treatment than in non-treated cats, indicating that the treatment had benefits in addition to changing emotional valence. Rewards may cause dopaminergic neuron activation (DA), particularly when greater than expected, producing dopamine associated with a feeling of pleasure [58]. Rewards can be anything that an animal wants and DA can be triggered by the auditory, visual, tactile, olfactory, and gustatory stimuli of foods [59]. In cats, there are also dopamine receptors in the gastrointestinal tract, where IgA is secreted [60], and DA increases blood flow to the mucosa-submucosa, inducing a pronounced mucosal vasodilatation up to 400% [61]. Thus, acquiring a desired resource, be it food or exiting the cage, may facilitate secretion of IgA. All aspects of the treatment were potentially rewarding for the cats. Hence, it is not clear which aspect may have facilitated contentment. It has been proposed that giving cats a puzzle feeder may be similarly stimulating and rewarding [62]. In addition, simply increasing the level of activity can stimulate IgA production [63]. The training session had several components, one of which was increased moderate exercise. Most cats, after a few sessions, jumped out of the cage and walked to the training room. The social aspect of the training session (the presence of a familiar human) may also have contributed to increased S-IgA. In humans, looking at a picture of a favourite person can increase IgA [64]. Conversely, frustration due to lack of access to resources, such as education and opportunity for social advancement, has been associated with elevated Epstein-Barr virus antibody titer and diastolic blood pressure [65]. Control cats were more likely to develop upper respiratory disease over the course of the study and to develop it significantly sooner than treated cats. Elevated S-IgA has consistently been reported to protect against URD in many species [66-69], and IgA deficiency has been associated with persistent symptoms of URD [70-72]. On the other hand, in a study of IgA knockout mice (IgA-/-) generated by gene targeting, IgA was not required for prevention of influenza virus infection and disease, indicating that other Ig’s may serve to decrease susceptibility.
to URD [73]. Pacing, escape attempts [27], and persistent meowing [28, 29] have been observed in caged cats and may be indicative of frustration.

4.1 Limitations of study

Samples were small thus the results were not definitive; however the results have important implications for hypothesis generation. In addition, our selection of cats frustrated upon admission may not be representative of the actual occurrence of these emotional states in cats as it is likely that frustration may occur more often after extended time at the shelter.

4.2 Conclusions

Cognitive enrichment, including learning a new task reinforced by an appetitive reward, together with time out of the cage in the company of a familiar human, were shown to induce and sustain positive affect in cats and were also associated with increase in the production of intestinal immunoglobulin A and lower incidence of upper respiratory disease in shelter cats.

References


Chapter 5: Effect of positive interaction with humans on mucosal immunity, incidence of respiratory disease and emotions in shelter cats.

Abstract

The link between positive affect and health has been demonstrated in many species. To examine the effect of sustained contentment on respiratory health in shelter cats, 96 cats were selected from 250 entering a shelter and classified as Anxious, Frustrated or Content using the Queensland Cat Wellbeing Index (UQ-ICW). Access of the cats to humans was controlled. Cats were either provided with positive human interaction such as petting, playing, and grooming in four 10 min sessions/d for 10 days or were exposed to a human standing in front of the cage with eyes averted for the same period. A mood score was assigned each day using UQ-CWI indicators to examine changes in emotional state, and the response to each treatment was classified as negative (involving aggression or retreat) or positive (calm or friendly). Treated cats were more likely to remain Content (Incident Rate Ratio: 1.93, Confidence Interval: 1.01-3.68, \( P < 0.0001 \)) and less likely to be rated as Anxious or Frustrated than Control cats (IRR: 0.61, CI: 0.42-0.88, \( P = 0.007 \)). Feline IgA (S-IgA), quantified in faeces by ELISA techniques, was greater for Treated (7.28 ±0.7 loge µg/g) than Control cats (6.74 ±0.5 loge µg/g, \( P < 0.0001 \)). Within the Treatment group, it was greater for cats that were rated Content throughout (7.52 ±0.5 loge µg/g) compared to cats that became Frustrated (7.24 ±0.3 loge µg/g) (\( P < 0.001 \)). S-IgA values were also greater in cats that responded positively than negatively to treatment (7.5 ±0.5 and 7.16 ±0.4 loge µg/g, respectively) (\( P = 0.02 \)). Infectious status was determined upon admission and on days 4 and 10 using combined conjunctival and oropharyngeal swab specimens tested by quantitative real-time PCR for feline herpesvirus type 1 (FHV-1), feline calicivirus (FCV), *Mycoplasma felis, Chlamydophila felis,* and *Bordetella bronchiseptica.* A larger proportion of Control than Treated cats shed pathogens overtime (Control 22%, 36%, 61%; Treated 35%, 26%, 32% on d 1, 4 and 10, respectively; \( P = 0.006 \)). The onset of upper respiratory disease was determined by veterinary staff based on clinical signs including ocular or nasal discharge. Control cats were more likely to develop URD overtime than Treated cats (HR 2.94, CI: 1.30-6.67, \( P = 0.01 \)). Cats that responded positively to gentling had a lower incidence of URD than negative responders (\( P = 0.02 \)). We conclude that the provision of positive human interaction to shelter cats can facilitate sustained contentment, enhance secretion of S-IgA, and reduce incidence of URD.

Keywords: Positive human interaction, Secretory Immunoglobulin A, Respiratory disease; Shelter cats.
1.0 Introduction

In humans, positive affect has been linked to a lower susceptibility to the common cold and influenza [1]. In cats, emotional stress is a risk factor for recrudescence of latent infections (e.g. Feline herpesvirus) [2], and chronic stress has been linked to an increased susceptibility to upper respiratory disease (URD) [3]. Conversely, cats showing low behavioural indicators of stress were found to have a lower rate of URD [3]. The mucosal immune system is the first line of defence against pathogens causing URD which are found in high concentration on shelter equipment, hands and clothing of caretakers [4]. Mucosal antibody -secretory immunoglobulin A (S-IgA), forms a glue-like substance in the mucosae of the nose, mouth and intestinal tract which prevents penetration of pathogens through the epithelial wall [5]. Stimulation of S-IgA is the underpinning of research for the development of effective intranasal vaccination for cats [6-8]. Conversely, IgA deficiency is characterized by recurrent signs of URD in adult humans [9], children [10] and dogs [11]. Similarly to cell mediated immunity [12]; the mucosal immune system is affected by emotional states. Regulation of IgA is affected by both acute and chronic stress [13], and by relaxation [14]. Dogs from private homes entering an army kennel for training initially experience an increase in salivary IgA on day 1, followed by decrease overtime [15]. Animal models indicate that the immune system can be manipulated by behavioural modifications [16-18]. In humans, IgA secretion can be stimulated with the aid of relaxation therapy [19], and increased IgA, in turn, has been shown to result in lower incidence of upper respiratory disease (URD) [1, 14, 20-22]. Feline IgA has been quantified in serum [23], saliva [24] and faeces [25]. IgA secretions in cats are most prominent in intestinal fluid compared to other body fluids [26], and can be measured non-invasively in faeces. The notion that facilitating positive affect in cats may reduce risk of respiratory disease in shelter cats is reflected in current guidelines and standards for the care of animals in shelters [27].

Newberry and colleagues proposed that enhancing emotional wellbeing should hold the same significance as other aspects of animal care, such as nutrition and veterinary care. In humans, it is believed that social support (positive human interactions) facilitates coping in times of stress and decreases susceptibility to disease [28]. Even looking at a photograph of a favourite person has been found to be sufficient to raise IgA level [29]. In shelter dogs, positive human interactions, including petting, grooming, and walking with a familiar person, reduces cortisol and heart rate [30-32]. Further, in dogs subjected to an aversive stimuli, the presence of a familiar human was associated with lower heart rate than the absence of that person [33]. Cats may similarly benefit from such interactions. For example, laboratory cats prefer interactions with humans compared to playing with
toys [34]. The effect of positive human interaction, with a familiar human, on S-IgA and susceptibility to URD has not been examined in cats. In this study, we hypothesize that positive human interaction may help cats sustain a state of contentment and stimulate S-IgA, which may reduce the incidence of URD.

2.0 Method.

This study was approved by the University of Queensland Animal Ethics Committee (CAWE/231/10). This study was part of a larger research project designed to examine the effects of behavioural interventions on cats rated as anxious, frustrated or content upon admission (See Chapter 2 for detailed method).

In brief, the study took place at the Vancouver Branch of the British Columbia Society for the Prevention of Cruelty to Animal (BC SPCA, Vancouver, Canada). Cats were either surrendered by their owner or brought in as strays by a humane officer between May and November 2010. Age was either provided by the owners or estimated by shelter staff and was categorized as juvenile (6-12 months), adult (1-7 years) or senior (>8 years). Upon admission, an animal health technician conducted a physical examination. Combined conjunctival and oropharyngeal swab specimens were collected before vaccinating (Fel-O-Guard+3 Boehringer Ingelheim Ltd., Canada) and deworming (Strongid® T. Pfizer, Canada) the cats. Swabs specimens from the same sites were also performed on days 4 and 10. Samples were tested by quantitative real-time PCR [35] for feline herpesvirus type 1 (FHV-1), feline calicivirus (FCV), Mycoplasma felis, Chlamydophila felis, and Bordetella bronchiseptica. Stools were collected daily, as available, and total feline faecal IgA was quantified by enzyme-linked immunosorbent assay (ELISA). Following examination, cats were transported in a small wire cage covered with a towel to the study ward and placed in a cage (76 x 76 x 71 cm) furnished with a litter box, food and water bowls and a towel for bedding. Feed was provided twice daily at 07:00 and 17:00 h (Science Diet, Hill's Pet Nutrition, Inc. ®/™, Vancouver. Canada) and fresh water was provided ad libitum. Cages were cleaned daily by removing all waste, changing bedding, and wiping walls with a clean cloth soaked in 1% antiseptic solution (Virkon®, Antec International Limited, Sudbury, UK).

2.1 Behavioural observation upon admission (Day 0)

A 1 h real-time video observation commenced as soon as the cat was placed in the cage. This was followed by a Human-Approach Test, adapted from an existing test [36] as follows: Step 1: the experimenter stood in front of the cage without any interaction, such as eye contact or verbal
greeting (2 min); Step 2: the experimenter talked to the cat using a high-pitched gentle tone and had some eye contact, but with eyes half closed) (1 min); Step 3: the same procedure was repeated with the door open, followed by an approach of the hand so that it was near the cat (2min). If the cat attempted to attack (bite or paw strike with hiss and/or growl) the door was closed immediately.

Cats were rated as Content if they engaged in one or more of the following behaviours: sitting quietly at the front of the cage, eating, grooming, sleeping or resting in a relaxed body posture such as lying on the side or ventrally recumbent with head held high, and if they showed friendly calm behaviour during the Human-Approach Test. In addition, the Content rating was only given if there was absence of hiding, flat posture, defensive retreat, and persistent meowing or attempts to escape.

2.2 Rating of emotional states each day (mood)

We measured changes in mood, an emotional response lasting more than a few hours [37], overtime based on focal sampling of behaviour from the videorecord. Sampling was for 10 min per h over the entire 10 d. Rating of daily mood was done according to behavioural indicators of Frustration and Contentment identified in a previous study [38]. The University of Queensland Indices of Cat Wellbeing (UQ-ICW) was developed in our previous study (See Chapter 2 for a detailed description) Some additional behaviours seen in the current study were added to the ethogram (Table 1): bar biting, oversleeping (90% of the day and difficult to wake up), lack of interest in surroundings and people, and absence of retreat or fear behaviour.

Application of UQ-ICW in this study

To assess emotional states per day for 10 days, focal sampling of behaviour was done using the videorecord (10 min per hour) for a total of 240 min per day. Rating for day 1 started at hour 2 and was therefore based on 230 min. Video recordings were coded by the experimenter (NG).

Anxious

Per 24 hour period, a cat was scored as Anxious if it engaged in Anxiety behaviour (Table 1) for a minimum of 192 minutes out of 240 minutes of observation. In addition, the cat was never observed sleeping while lying on side, calmly walking around while in an upright position or sitting in an upright posture at the front of the cage.
**Frustrated**

Per 24 hour period, a cat was scored as Frustrated if it engaged in Frustration behaviour (Table 1) for a minimum of 24 minutes out of 240 minutes of observation. In addition, the cat was never observed engaging in Anxiety behaviours or sleeping while lying on side.

**Content**

Per 24 hour period, a cat was scored as Content if it engaged in Contentment behaviour (Table 1) for a minimum of 192 minutes out of 240 minutes of observation. In addition, the cat was never observed engaging in Anxiety behaviours or persistent meowing, scanning, repetitive or escape behaviour.

*The experimenter conducting the assessments (NG) was not blind to the study. As the coding of behaviour and rating was done by video following the experiment, the experimenter was familiar with all cats including their grouping (Control or Treated) and cage number.*
Table 1: UQ-ICW behavioural indicators for the rating of emotional states in cats

<table>
<thead>
<tr>
<th>Anxiety behaviour &gt; 80% of observation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flat</td>
</tr>
<tr>
<td>Low body posture when lying down, sitting or standing for locomotion</td>
</tr>
<tr>
<td>Freeze</td>
</tr>
<tr>
<td>Tense, immobile flat body posture, eyes wide open Remains completely immobile, body and head flattened to floor</td>
</tr>
<tr>
<td>Hide</td>
</tr>
<tr>
<td>Hides under bedding, litter tray, behind or in litter box</td>
</tr>
<tr>
<td>Startle</td>
</tr>
<tr>
<td>Sudden retreat or flatten to back of cage</td>
</tr>
<tr>
<td>Crawl</td>
</tr>
<tr>
<td>Slow locomotion keeping body and head lowered</td>
</tr>
<tr>
<td>Retreat</td>
</tr>
<tr>
<td>Retreats to the back of the cage or flattens when humans approach the cage</td>
</tr>
<tr>
<td>Self-maintenance</td>
</tr>
<tr>
<td>Grooming, feeding and locomotion are inhibited. Particularly do not occur in the presence of humans</td>
</tr>
<tr>
<td>Absent</td>
</tr>
<tr>
<td>Sleep or rest with lie on side, Sit.front, walk.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Frustration behaviours &gt;10% of awake time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meow</td>
</tr>
<tr>
<td>Persistent meow not related to anticipation of food</td>
</tr>
<tr>
<td>Escape bouts</td>
</tr>
<tr>
<td>Very active and includes one or more behaviours performed persistently or frequent in repeated bouts and occurs mainly at the front of the cage: On hind limbs, pawing wall/floor, paw through door, push on door or latch, hanging on cage door with body inverted</td>
</tr>
<tr>
<td>Scan</td>
</tr>
<tr>
<td>Visual scanning of the cage</td>
</tr>
<tr>
<td>Push</td>
</tr>
<tr>
<td>Hits or throws objects around the cage in a destructive manner using head, body or paws (not related to play.) Spills food bowls, and litter. May push on cage door with head, paws or body.</td>
</tr>
<tr>
<td>Pace</td>
</tr>
<tr>
<td>Repetitive and fast walking back and forth in the cage particularly by the door</td>
</tr>
<tr>
<td>Redirected aggress.</td>
</tr>
<tr>
<td>Quick bite, swipe or scratch during interaction with human without defensive vocalizations, body posture or retreat. May occur in bouts during otherwise friendly interactions</td>
</tr>
<tr>
<td>Bar biting*</td>
</tr>
<tr>
<td>Bites or lick cage bars persistently or in repeated bouts</td>
</tr>
<tr>
<td>Absent</td>
</tr>
<tr>
<td>All Anxious behaviours and lie on side</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contentment behaviours &gt; 80% of observation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep/rest</td>
</tr>
<tr>
<td>Relax body posture and neck, eyes closed or semi closed</td>
</tr>
<tr>
<td>Lie on side</td>
</tr>
<tr>
<td>Laterally recumbent, body stretched, neck and ventral area exposed, limbs and tail extended</td>
</tr>
<tr>
<td>Front.sit</td>
</tr>
<tr>
<td>Sits at the front of the cage, calmly looking around</td>
</tr>
<tr>
<td>Groom</td>
</tr>
<tr>
<td>Licks fur or paws and rubs head with paws (without chewing or pulling coat)</td>
</tr>
<tr>
<td>Walk</td>
</tr>
<tr>
<td>High body posture, normal gait and pace to move around the cage (not on-going or repetitive)</td>
</tr>
<tr>
<td>Eat / drink</td>
</tr>
<tr>
<td>Takes food or water into mouth and ingest. Particularly in the presence of humans. Does spill food or water around the cage</td>
</tr>
<tr>
<td>Friendly</td>
</tr>
<tr>
<td>Approaches human at front of cage in amicable manner.</td>
</tr>
<tr>
<td>Absent</td>
</tr>
<tr>
<td>All Anxious behaviour, persistent meowing, scanning, repetitive or escape behaviour</td>
</tr>
</tbody>
</table>

*bar biting was not included in the UQ-ICW (not validated with cortisol or S-IgA) but was added to this study as it was frequently observed together with other Frustration behaviours.*
2.3 Treatment procedures

As they were received cats were allocated at random to Treatment or Control groups. Treatment (positive human interaction) was provided 4 times per d for 10 min each time (commencing at 06:00 h, 11:00 h, 16:00 h and 20:00 h) by the experimenter (NG) or one of two trained volunteers. Cats received treatment in the same order each time; however, the exact time of treatment depended on the number of cats to be treated each day. The treatments aimed to mimic the interactions usually provided by volunteers in this shelter and were as follows: Step 1: Verbal greeting using a high-pitched gentle tone (30s), with the door closed. Step 2: The door was opened and an approach with the hand was offered for the cat to sniff. Step 3: The experimenter sat on a chair next to the cage. Step 4: Cats were petted without restriction as to which area of the body was touched. Step 5: The experimenter placed a brush and a felt string in the cage. Cats that showed interest in either by sniffing or rubbing on the object were brushed or allowed to play with the string. Cats in the Control group were exposed to a human standing in front of the cage with eyes averted for the same period, to ensure that both groups were exposed to humans for the same total time over the study, with the difference between groups being only in the provision of interaction with the human.

2.4 Rating of response to treatment

The response of cats to treatment was rated as positive or negative, according to behavioural indicators outlined in Table 2. Behavioural responses were noted immediately after each treatment.

Table 2: Behavioural indicators used for the classification of responses to positive human interaction.

<table>
<thead>
<tr>
<th>Rating</th>
<th>Description of responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>a) Defensive aggression: onset of hissing, growling and paw strike.</td>
</tr>
<tr>
<td></td>
<td>b) Defensive retreat: flattens body and ears, freezes or retreats.</td>
</tr>
<tr>
<td>Positive</td>
<td>a) Calm: relaxes body, lowers the head when petted between ears, raises the head when petted on the chin. Absence of defensive aggression or retreat.</td>
</tr>
<tr>
<td></td>
<td>b) Friendly: stands close to, or walks to the experimenter; or remains in a sitting or lying down posture, rubs themselves on the experimenter’s hands, maintains a relaxed body posture; may also walk to the food bowl and eat during treatment.</td>
</tr>
</tbody>
</table>

2.5 Statistical analysis

All tests were assumed to require an alpha of 0.05 for significance.
2.5.1 Statistical analysis of cat characteristics

Fisher’s exact test was used to determine if there was a significant difference in cat characteristics at the time of enrolment.

2.5.2 Effect of treatment on daily mood and mood change overtime

A Poisson regression analysis was used to compare the daily mood rating for Treated and Control cats that had been rated as Content on arrival. For all Poisson regression analyses, an incidence rate ratio (IRR), confidence interval (CI) and corresponding p-value are reported. The response variable was the number of cats rated as Content, Frustrated or Depressed each day and the explanatory variables were Treated/Control group and day. The poisson model was used in preference to other count models, such as negative binomial or zero-inflated models, because the response variable was not over-dispersed and did not have an excessive number of zeros. Generalized estimating equations (GEE) were used to determine if the cat characteristics were significant predictors of daily mood scores, these being appropriate when there are correlations between observations (in this case days for each of the cats).

2.5.3 Influence of mood and response to treatment on S-IgA levels

A Student’s t-test was used to determine if there was a mean difference in the number of stools between Treated and Control cats. S-IgA values were loge transformed to achieve a normal distribution. A GEE was used to determine if there was a significant difference in S-IgA levels depending upon time (day) in the shelter. The GEE was then used to determine differences in S-IgA levels between the Treated and Control groups and between responses to treatment (positive, negative) within treatment groups and according to age, source and gender. Mood ratings on days for which there were no available stools (within 24 hours of rating) were removed from the analysis.

2.5.4 Incidence of shedding and URD by group

Fisher’s exact test was used to determine if the rate of shedding on each of the days, as well as the incidence of URD, were significantly different between Treated and Control groups. GEE was used to determine if the rate of shedding changed overtime.

To determine if the time to develop URD was different between Treated and Control cats, a t-test was utilized. Fisher’s exact test was used to determine if the incidence of URD differed between Treated and Control groups (Odds ratio, CI and corresponding p-value are reported). A Cox-Proportional Hazards model was used to compare the incidence of URD in Treated and Control
groups overtime. Additionally, GEE were used to determine if S-IgA levels and incidence of URD differed according to responses to treatment (positive or negative), and Fisher’s exact test was used to determine if the incidence of URD differed according to age, source, sex, and sterilization status.

3.0 Results

3.1 Baseline cat characteristics

Cats were equally distributed to the Treated and Control groups for the age (seniors, adults, and juveniles), gender (male, female), and source (owner-surrendered, strays) variables (P >0.05; Table 3). There was no difference response to the Human Approach Test (no analysis performed), all cats had responded positively.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>51</td>
</tr>
<tr>
<td>Adult</td>
<td>30</td>
<td>61.22</td>
</tr>
<tr>
<td>Juvenile</td>
<td>12</td>
<td>24.49</td>
</tr>
<tr>
<td>Senior</td>
<td>7</td>
<td>14.29</td>
</tr>
<tr>
<td>Intact</td>
<td>20</td>
<td>40.82</td>
</tr>
<tr>
<td>Neut.</td>
<td>29</td>
<td>59.18</td>
</tr>
<tr>
<td>Male</td>
<td>24</td>
<td>48.98</td>
</tr>
<tr>
<td>Female</td>
<td>25</td>
<td>51.02</td>
</tr>
<tr>
<td>Owner surrendered</td>
<td>14</td>
<td>28.57</td>
</tr>
<tr>
<td>Stray</td>
<td>35</td>
<td>71.43</td>
</tr>
</tbody>
</table>

3.2 Daily mood and the effect of mood on response to treatment

Over the 10 days of treatment, Treated cats were more likely to sustain a Content mood than Control cats (IRR: 1.93, CI: 1.01-3.68, P <0.0001, Fig. 1). Specifically, the mood on 384 of 427 days of observation (89%) was rated as Content for Treated cats compared to 345 days of 413 (83.5%) for Controls. Control cats were more likely to experience negative affect (Anxiety or Frustration) than Treated cats (IRR: 0.61, CI: 0.42-0.88, P =0.007; Fig.2). Specifically, 68 out of 413 days of observation (16.5%) were rated as Anxious (2.1%) or Frustrated (14.2%) for Treated cats versus 43 of 464 days (10.1%) rated as Anxious (1%) or Frustrated (9%) for Controls.
3.3 Influence of mood and response to treatment on S-IgA levels

Age, source, gender, carrier status, and response to treatment did not predict daily mood score ($P > 0.10$). However, intact cats were more likely to be rated as Content, showing sustained contentment for 184/187 days, compared to neutered cats which showed it only on 211/234 days ($P = 0.007$).

There was a significant difference in the mean number of stools analysed for the two groups (Control $3.9 \pm 1.9$/cat, Treated $4.7 \pm 1.7$/cat; $P = 0.03$). There was a reduction in S-IgA level overtime.
in both groups (Controls $P =0.05$, Treated $P =0.06$, Fig.3). Treated cats had higher S-IgA levels (7.28 ±0.7 loge µg/g) than Control cats (6.74 ±0.5 loge µg/g, $P <0.0001$). S-IgA was higher for Treated cats that sustained contentment (7.52±0.5 loge µg/g) than for Treated cats that became Frustrated or Anxious (7.24±0.3 loge µg/g) ($P <0.001$). Treated cats that sustained contentment had significantly higher S-IgA (7.52 ±0.5 loge µg/g) than Control cats that also showed sustained contentment (7.01 ±0.7 loge µg/g) ($P <0.001$). Response to treatment (positive/negative) did not affect S-lgA levels ($P =0.18$). Overall, there was no significant difference in S-IgA attributable to source, gender or sterilization status ($P ≥0.14$). However, there was a trend for S-IgA concentration to be increased with age (Adult 6.65±0.6 loge µg/g, Juvenile 6.70±0.7 loge µg/g, and Senior 6.77±0.7 loge µg/g; $P =0.07$).

![Fig. 3: Generalised estimated equations comparing day effect on Mean loge transformed µg S-IgA/g (wet faeces) for cats in the Treatment and Control groups.](image)

3.4 Effect of treatment on URD

There were no differences in shedding rate upon admission between cats assigned to the Treatment and Control groups for all pathogens (Fisher’s exact test $P >0.05$). However, there was an increase in shedding overtime in Control cats (22%, 36% and 61% on days 1, 4 and 10, respectively), but not Treated cats (35%, 26%, 32% on days 1, 4 and 10, respectively) (GEE $P =0.006$).
Control cats were more likely to develop URD overtime compared to Treated cats (Fig. 4, HR: 2.94, CI: 1.30-6.67, \( P =0.01 \)). Response to treatment (positive/negative) was a significant predictor of URD (\( P =0.02 \), Table 4). Of the 49 cats in the control group, 17 cats (34.7%) developed URD. Of the 47 cats in the treatment group, 9 (19.1 %) developed URD. Thus there was a reduction of 15.6 % in URD in cats receiving treatments. There was no difference in the incidence of clinical URD according to source, age or gender (\( P >0.05 \)).

Table 4: Difference in URD status according to number and percentage of negative or positive responses to treatment.

<table>
<thead>
<tr>
<th>Responses</th>
<th>No URD</th>
<th>URD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
<td>0.48</td>
</tr>
<tr>
<td>Positive</td>
<td>317</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Fig. 4 Cumulative probability of developing URD overtime (40 days) in Treatment and Control groups.

4.0 Discussion

In this study, we tested the hypothesis that positive interactions with a familiar human could help cats experience sustained contentment and that this may reduce susceptibility to URD by stimulating secretion of IgA. The starting point for this study was based on our previous study
(Chapter 2) which showed that Content cats had significantly higher S-IgA values than Anxious and Frustrated cats. In both the previous studies and this one, Contentment was indicated by behaviours such as sitting at the front of the cage, lying on the side while resting or sleeping, friendly behaviour towards humans, normal patterns of feeding, grooming and locomotion, and the absence of defensive or restless behaviour. Of the 250 cats which took part in the larger research project, 38% were rated as Content upon admission, compared to 56% and 6% rated as Anxious and Frustrated (negatively valenced states), respectively; supporting the notion that entering an animal shelter induces stress in cats [39-41].

Our findings show that positive interactions with a familiar human facilitated sustained contentment, whereas depriving cats of such contact resulted in the onset of anxiety and frustration. Similar findings were reported in cats that were not provided with environmental enrichment, such as a hiding box [42]. The authors reported that some cats that were calm and content after one week at the shelter were hiding behind the litter box by week two, suggesting that some cats may not be able to sustain positive affect unless provided with some form of enrichment. In the current study, both Treated and Control cats experienced a decrease in S-IgA overtime, indicating that all may have experienced some emotional stress. However, S-IgA remained higher in Treated cats throughout the study, particularly for those with sustained contentment compared to those that became frustrated or anxious. Similarly in humans, sustained contentment (or happiness) is correlated with enhanced immune protection [43].

We found that the age, source or sex of the cats did not influence mood overtime; however, intact cats were more likely to sustain a positive mood (Contentment) than neutered cats. These results may have been influenced by previous lifestyle of intact cats most of which were strays. Dydall and colleagues had found that stray cats exhibited less stress related behaviours than those surrendered by their owners [44]. Conversely, McCobb [45] found no difference in adrenal output between in strays and owner-surrendered cats over 19 days at an animal shelter. It is possible that intact cats even when owned are given access to outside more so than neutered cats given them exposure to a wider range of experiences and possibly facilitating adaptation to the shelter. It has been reported that cats well-socialized to other cats adapt faster to the shelter environment [36] On the other hand, intact cats tend to roam over a wider territory and may therefore be expected to experience more frustration in response to confinement.
The positive effect of interactions with a familiar human was most evident when we compared S-IgA in cats with sustained contentment from both groups. Content cats in the treatment group had higher S-IgA values than Content cats from the Control group. In humans, a 10 min relaxation session is sufficient to increase S-IgA [19]. On the other hand, the presence of a human close to the cage without giving eye or vocal contact may have caused sufficient stress to inhibit secretion of IgA, even if not sufficient to alter mood overall. This was supposed to provide the control, of people being in the room but not positively interacting with the cat in question. In dogs, removal of a positive reinforcer, such as positive human attention, is commonly used as a means to extinguish an undesired behaviour [46], indicating the powerful effect that attention from a familiar person has on dogs. Laboratory cats used to receiving vocal interaction and petting during regular husbandry practices show an increase in adrenal output when the interaction is no longer provided [47].

Our hypothesis that sustained contentment may contribute to respiratory health in cats was well supported by the current findings. There were no differences in shedding rate among the two groups upon admission to the shelter, indicating that there were a similar number of cats in both groups with subclinical infections (although some carriers may not have been shedding at the time). However, there were 10% more shedders by day 4 and 29% more by day 10 among Control cats. According to Pedersen and colleagues [48], the onset of shedding shortly upon entering a shelter may indicate the presence of a subclinical infection, whereas the onset of shedding later may indicate an infection contracted at the shelter. Thus, we interpreted the differences in shedding rate on day 4 to be indicative of viral reactivation in cats with subclinical infections. It is well documented that stress is a major factor in reactivation of subclinical infections, particularly feline herpesvirus [2, 49]. Thus we may interpret the current data to mean that positive human interaction may prevent the onset of shedding in cats with subclinical respiratory infections. Further, the higher percentage of shedders by day 10 in the Control group may be interpreted to mean that the Treatment increased the cats’ resistance to the pathogens in the shelter environment. As it is difficult to completely eliminate pathogens from shelter equipment and from the clothing and hands of shelter staff [4], it is particularly important to increase the cats’ resistance to these pathogens.

Our hypothesis was also supported by findings that Treated cats had a lower prevalence of URD than Control cats. Although this study took place in an animal shelter, conditions were unusual. Few people were allowed access to the room and husbandry was consistent. In addition, the treatments were provided by the same person who, therefore, became familiar to the cat. Under usual shelter practices, cats are exposed to more stressors (noise, people traffic, inconsistent husbandry) and it is likely that
different people (usually volunteers) interact with the cats, which may reduce the effectiveness of this interaction. In dogs, heart rate during interaction with a human may vary according to familiarity with the petter and the context of the activity [50]. However, it has also been proposed that shelter cats may lose interest in interactions due to habituation to the presence of a human [51]. Thus, interactions with different people may be beneficial.

5.0 Conclusions

Positive human interactions provided by a familiar person helped cats to sustain positive affect, enhanced secretion of IgA, and reduced the prevalence of upper respiratory disease in the shelter environment under study.

References


51. Hoskins C: The effects of positive handling on the behaviour of domestic cats in rescue centres. MSc.: University of Edinburgh; 1995.
Chapter 6: Descriptive epidemiology of upper respiratory disease and associated risk factors in cats in an animal shelter situated in coastal western Canada.

(Formatted for the Canadian Veterinary Journal)

Abstract

Understanding risk factors for feline upper respiratory disease (URD) is important for veterinary health teams responsible for the control of disease in animal shelters. We examined 250 cats at an animal shelter in the coastal temperate region of Canada. We determined if characteristics such as age, source, sex, and sterilization status influenced shedding risk at intake, transmission of infection and development of clinical URD. Upon admission, 28% were positive for one or more infectious agent related to URD (determined by real time polymerase chain reaction, RT-PCR). Twenty-one percent were carriers of *Mycoplasma felis* (*M.felis*) and fewer than 3% were carriers of feline calicivirus (FCV), feline herpesvirus-1 (FHV-1) or *Bordetella bronchiseptica*. *Chlamydophila felis* and H1N1 virus were not detected. Carrier status was not affected by source, sex, sterilization status or age (*P* > 0.05). Viral and bacterial shedding increased by 9% and 11% respectively over the three sampling times (days 1, 4 and 10). Over 40 days post admission, the cumulative probability of developing URD was 2.2 times greater for strays than owner-surrendered cats (*P* = 0.02) and 0.5 times greater for neutered cats than for intact cats (*P*=0.03). Cats that were shedding at intake were 2.6 times more likely to develop URD than non-carriers (*P* < 0.002). Most at risk were cats with FHV-1 and *B. bronchiseptica* infections compared to non-shedding cats (*P*<0.01).

We concluded that shedders of FHV-1 or *B. Bronchiseptica* and stray or neutered cats were most at risk of developing URD in the shelter environment tested.

Key words: Animal shelter; Cats; Canada; Upper respiratory disease; Risk factors.
1.0 Introduction

Upper respiratory disease (URD) is the primary health issue reported in cats during their stay in animal shelters [1] and post adoption [2, 3]. In shelters, URD is an important cause of morbidity due in part to factors such as poor ventilation, stress induced immunosuppression and overcrowding which complicate management of disease [4]. URD is the primary health reason for euthanasia of kittens in animal shelters [5] and cats receiving treatment are subjected to extended periods of confinement with minimal human interaction [6]. URD outbreaks are a common problem in animal shelters [7], which together with the day to day management of sick cats represents a significant financial burden for humane organizations.

The feline respiratory disease complex encompasses a variety of pathogens. Feline herpesvirus-1 (FHV-1) and feline calicivirus (FCV) are believed to be responsible for most cases of URD in animal shelters followed by *Mycoplasma felis* (*M.felis*), *Bordetella bronchiseptica* (*B. bronchiseptica*) and *Chlamydophila felis* (*C.felis*) [8]. In March 2009, a new human influenza A H1N1 virus emerged in Mexico and the United States [9]. From fall 2009 to early 2010 in the United States there were several reports of H1N1 influenza virus in animals, two of which were reported in domestic-owned cats believed to have contracted the virus from their owners [10]. FHV-1, which has a prevalence range of between 0.2 and 33 % in household cats [11] has been reported at rates between 63% and 84% in shelter cats in South Korea [12], Belgium [13], and California, USA [14]. FCV, which is present in about 8 percent of household cats [14], is believed to affect about 40% of shelter cats [15]. Stray cats admitted to shelters are a known source of these pathogens [16] and there are equivocal results as to whether age, gender and sterilization status are potential risk factors [14, 17, 18]. However, scientists agree that identifying the characteristics of cats at greater risk for developing URD is critical to the management of the disease in animal shelters [7, 19, 20].

Despite a substantial body of knowledge on prevalence of URD and associated risk factors in animal shelters worldwide, the epidemiology of URD in Canada, particularly in the coastal temperate climatic region which exists in British Columbia, has not been examined. The Canadian Federation of Humane Societies [21] estimates that there are about 150 Humane Societies/SPCAs across Canada, each managing many shelters, in addition to many private rescue organizations and animal control agencies. The present study examined the prevalence of subclinical upper respiratory infections in cats upon admission to an animal shelter, and the risk factors associated with the subsequent spread of infection and development of URD overtime.
2.0 Materials and Methods

The study took place at the Vancouver Branch of the British Columbia Society for the Prevention of Cruelty to Animal (BC SPCA), Canada between May and November 2010. It was part of a research project to examine the emotional and immunological changes in anxious, frustrated or content cats when provided with behavioural interventions. During the first 10 days of the study, cats were housed in a pre-adoption housing unit with limited access to the public.

Cats were housed individually in stainless steel cages (76 x 76 x 71 cm) furnished with litter boxes, stainless steel food and water bowls and bedding. Age-appropriate food (Hill's Pet Nutrition, Inc. ®/™) and water was provided twice per day (07:00 h and 17:00 h). Windows provided natural light and temperature was maintained at 20 (± 2) degrees Celsius. The shelter has six separate housing areas with a maximum capacity to house 120 cats. The facility also includes an isolation area for sick cats and in-house medical staff at the on-site veterinary hospital. The shelter followed strict biosecurity measures, including spot cleaning of cages daily (removing debris and wiping cages with a clean cloth dipped in 1% Virkon® solution) and disinfection of cages between cats with a 2%Virkon® solution. Animal care staff did not wear gloves or protective gowns during cleaning of cages but washed their hands with a foaming alcohol handrub (Microsan™ Encore. DEB, Canada) following contact with an animal. Cats with observed clinical signs of URD, such as sneezing, were immediately transferred to an isolation ward and received medical care.

2.1 Animals

Two-hundred and fifty cats that were either surrendered by their owner or brought in as strays by a humane officer were enrolled in the study. Age was either provided by the owners or estimated by shelter staff, and categorized as juvenile (6 to 12 months), adult (1 to 7 years) or senior (> 8 years). Of the 250 cats, 50% were receiving one of three behavioural interventions to reduce emotional stress (See Chapters 3, 4 and 5).

2.2 Bacterial and Viral sampling

Swabs were obtained at intake and subsequently on days 4 and 10. The procedure was carried out by one of three Registered Animal Health Technicians using PCR swabs according and IDEXX procedure. A sterile swab was rolled on the medial conjunctiva and another on the posterior oralnasal pharynx. The swabs were placed into individual sterile transport tubes (ST RPLEX, Etobicoke, Ontario. Canada) refrigerated and submitted for real-time polymerase chain reaction (PCR) testing within 8 h. After the first swab, cats were vaccinated with a modified live vaccine.
Chapter 6

(Fel-O-Guard+3 Boehringer Ingelheim Ltd., Canada) and dewormed (Strongid® T). Cats with observed clinical signs of URD, gingivitis or injury at admission were not included in the study. Samples were analysed for feline herpesvirus 1 (FHV-1), feline calicivirus (FCV), Chlamydophila felis (C. felis), Mycoplasma felis (M. felis), Bordetella bronchiseptica (B. bronchiseptica) and influenza virus H1N1 by real-time polymerase chain reaction (RT-PCR) assays, that were based on IDEXX’s oligonucleotides and protocols [22]. Each test used a fluorescent probe that matched with a unique segment of the organism’s DNA or cDNA to ensure high specificity and sensitivity. RT-PCR was performed with standard primer and probe concentrations using the Roche LightCycler® 480 Probes Master mastermix (Roche Applied Science, Indianapolis, USA) and default cycling conditions on a Roche LC480 instrument and 384-well plate configuration.

3.0 Statistical Analysis

Prevalence of subclinical infections was calculated as the number of cats without clinical signs of URD that were PCR positive for FHV-1, FCV, C. felis, M. felis, B. bronchiseptica upon admission, divided by all cats included in the study (n=250). Multivariate logistic regression was used to examine the effects of sex (male versus females), source (owner-surrendered (OS) versus strays), sterilization status (intact versus neutered), age (juveniles, adults and seniors) and carrier state (shedding versus not shedding at intake for each specific pathogen) on the incidence of URD. To determine the influence of the individual pathogens (FCV, FHV-1, B. bronchiseptica, C. felis and M. felis) on the prevalence of URD, an ANCOVA was performed where the covariates included were sex, source, sterilization and age. These analyses were run on only a subset of the 250 cats which included carriers of the particular pathogen and non-carriers of all pathogens (carriers of other pathogens were excluded from the analyses). Cats could be carriers for more than one pathogen, so some cats were included in more than one analysis. The p-values derived from a model adjusted for are reported for these analyses.

A Kaplan Meier survival analysis [23] compared the percentage risk of developing URD by day 7, 14, 21 and > 30 (maximum of 40 days) according to demographic characteristics such as carrier status, age, source, sex and sterilization status. A Cox regression analysis using the survival package in R statistical software was used to determine if the risk of contracting URD overtime was significantly affected by these characteristics. All analyses were performed with R version 2.10.1.[24].
*In this study cats that were shedding (PCR positive) were labelled carriers and those not shedding were labelled non-carrier. However, cats that were not shedding may have been carriers but were not shedding at the time of swabbing.*

4.0 Results

4.1 Shedding rate at intake and overtime

At intake, 28% (n = 69) of cats were carriers for one or more pathogens. Of the positive samples 22%, 2.8%, 2.0% and 2.4% were positive for *M. Felis*, FCV, FHV-1 and *B. bronchiseptica* respectively. Co-infections with *M. felis* were identified for *B. bronchiseptica* samples and FCV for 3 and 1 of the samples respectively. Of the samples obtained at admission, all were negative for *C. felis* and H1N1 virus. Risk of being a carrier was not significantly affected by sex, source, sterilization status or age (Fisher’s exact text \( P > 0.05 \)) (Table 1). Subsequent swabs (days 4 and 10) showed an increase of 9% and 11% over the 10 days in viral and bacterial infections, respectively. FCV (1%), *B. bronchiseptica* (3%) and *C. felis* (1%) showed a lesser increase than FHV-1 (8%) and *M. felis* (10%) (Table 2). All cats remained negative for H1N1 virus throughout the study.

Table 1: Carrier status for FCV, FHV-1, *M. felis* and *B. bronchiseptica* (*B.b*) in cats (n = 250) on intake to the shelter, presented according to the following cat characteristics (\( P > 0.05 \)): sex, source, sterilization status and age category.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total</th>
<th>FCV</th>
<th>%</th>
<th>FHV-1</th>
<th>%</th>
<th>M. felis</th>
<th>%</th>
<th>B. b</th>
<th>%</th>
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<tr>
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<tr>
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<tr>
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<td>0</td>
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<td>33</td>
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</table>

Table 2: Prevalence of shedding of various viruses* and bacteria from ocular and pharyngeal swabs of shelter cats (n = 250) performed at intake (before vaccination, day 1), at day 4 and day 10.
4.2 Risk factors associated with the development of URD

As shown in Table 3, sex was a not significant factor for the development of URD ($P = 0.43$). Neutered cats had a greater prevalence of URD (39%, $n = 57$) than intact cats (26%, $n = 27$) ($P = 0.03$). When all pathogens were considered together, the risk of developing URD was 2.6 times greater for carriers (53%, $n = 47$) than for non-carriers (23%, $n = 37$) ($P < 0.002$). All FHV-1 carriers (100%, $n = 5$) developed URD compared to cats without subclinical infections (28%, $n = 51$) ($P < 0.002$), whereas the risk was not significantly greater ($P = 0.46$) for FCV carriers (43%, $n = 7$). Although prevalence of *M. felis* shedding (21%, $n = 54$) was greater than for all other pathogens combined, the risk of developing URD was not significantly greater for those cats (Odds Ratio 1.6) ($P = 0.29$). The sample of cats with subclinical *B. bronchiseptica* infection upon admission was small ($n = 6$). However, these cats were significantly more likely to develop URD than non-carriers ($n = 181$) (Odds Ratio 12.6; $P = 0.01$).
Table 3: Multivariate* associations of upper respiratory disease in shelter cats (n = 250) with characteristics and shedding status.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>cats without URD</th>
<th>%</th>
<th>cats with URD</th>
<th>%</th>
<th>Odds Ratio</th>
<th>95% Confidence limits for the odds ratio</th>
<th>p-value</th>
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<td>130</td>
<td>72</td>
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<tr>
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<td>61</td>
<td>21</td>
<td>39</td>
<td>1.62</td>
<td>0.81 3.20</td>
<td></td>
</tr>
</tbody>
</table>

*Data were analysed by analysis of covariance, adjusting for the following covariates: sex, source, sterilization status and age

4.3 Cumulative risk of developing URD overtime

Cats in the study were at the shelter from two to 191 days. The median length of stay before cats exhibited clinical signs of URD, were adopted, redeemed or euthanized was 14 days. Median time to URD for carriers of FHV-1 and FCV was 6 and 2 days, respectively, compared to 11 days for non-carriers of these two viruses. Median time to the development of URD for cats with a subclinical *B. bronchiseptica* infection at intake was 8 days compared to 12 days for non-carriers. As shown in Table 4, there was no significant effect of sex or age class on the cumulative risk of developing URD overtime. The cumulative risk of developing URD was significantly greater for strays than for OS cats [Hazard Ratio = 2.46 (1.48-4.09), P = 0.001]. The likelihood of developing URD was 17% greater for strays than for OS cats after 30 days (Figure 1). Similarly, the cumulative risk of developing URD was greater for neutered than for intact animals [Hazard Ratio = 0.43 (0.23-
The likelihood for onset of clinical URD was 9% higher than for intact cats by day 30 (Figure 2). Cats with subclinical infections had the greatest cumulative risk for URD (Hazard Ratio = 2.39 (1.44-3.99), P <0.001) compared to non-carriers. By day 7, 29% of carriers were at risk of developing URD compared to 2.7% for non-carrier cats. By day 14, the risk had increased to 46% for carriers compared with 13% for non-carriers. This trend continued into week three with 55% carriers versus 28% non-carriers and beyond 30 days with 76% carriers versus 41% non-carriers (Figure 3).

Table 4: Cox Proportional Hazards Models and significance of the survival analysis for each of the demographic characteristics.

| Characteristic | N  | Day 7 % | Day 14 % | Day 21 % | Day >30 days % | Hazard Ratio (CI) | Pr (>|Z|) |
|----------------|----|---------|---------|---------|---------------|------------------|----------|
| **Sex**        |    |         |         |         |               |                  |          |
| Female         | 138| 9.5     | 23      | 38      | 45            | 1.17 (0.72-1.93) | 0.52     |
| Male           | 112| 13      | 31      | 48      | 63            |                  |          |
| **Source**     |    |         |         |         |               |                  |          |
| CS             | 125| 7.3     | 19      | 33      | 45            | 2.46 (1.48-4.00) | 0.001    |
| Stray          | 125| 16.3    | 36      | 50      | 61            |                  |          |
| **Sex status** |    |         |         |         |               |                  |          |
| Neutered       | 148| 15      | 30      | 45      | 53            | 0.43 (0.23-0.78) | 0.01     |
| Intact         | 102| 7       | 15      | 35      | 44            |                  |          |
| **Age**        |    |         |         |         |               |                  |          |
| Adult          | 50 | 14      | 22      | 40      | 44            | 1.85 (0.94-3.64) | 0.07     |
| Juvenile       | 134| 19      | 27      | 44      | 63            | 1.19 (0.67-2.10) | 0.56     |
| Senior         | 66 | 11      | 30      | 45      | 60            |                  |          |
| **Carrier state** |   |         |         |         |               |                  |          |
| Non-carrier    | 162| 2.7     | 13      | 28      | 41            | 2.39 (1.44-3.99) | < 0.001  |
| Carrier        | 88 | 29      | 46      | 55      | 76            |                  |          |
Fig. 1 The effect of source of cat on cumulative probability of developing clinical URD overtime.

Fig. 2 The effect of sterilization status of cat on cumulative probability of developing clinical URD overtime.
Discussion

The primary objective of this study was to determine the risk factors associated with subclinical upper respiratory infections in cats entering a Canadian shelter (BC SPCA, Vancouver, Canada) and those associated with subsequent onset of clinical URD. Pharyngeal and ocular swabs were obtained from 250 cats upon admission and subsequently on days 4 and 10. FCV and FHV-1 are believed to be the most common pathogens in household and shelter cats [3, 7]. The 2.8 % shedding rate for FCV in cats entering this shelter was lower than prevalence reported in a similar survey in a California shelter (11%) [25]. Kittens are known to be particularly vulnerable to FCV [26]. Our findings showed no effect of age on vulnerability to FCV. In concordance with findings by Pedersen et al., [25], FCV did not increase overtime (1% increase by day 10). In this study, cats were housed singly, thereby minimizing cat-cat transmission of the virus. FHV shedding was also low upon admission (2%). Although, FHV-1 shedding increased overtime (8%), prevalence overall was much lower than that reported in that study (>50% after one week). This pathogen may be a particular risk for shelter cats as latent infections can be reactivated in response to stress and cause recrudescent clinical disease [27]. *M. felis* are normal commensal organisms of the upper respiratory tract, but some species have been implicated in clinical URD in both household [28] and shelter cats (≥ 47%) [29, 30]. Our findings indicated a high prevalence of *M. felis* upon admission (21%) and showed the most important increase overtime (10%) in cats that remained healthy. Overall the incidence of clinical URD in this Canadian shelter (34%) was similar to rates
reported in North-eastern US shelters (33%) [5] and lower than rates reported in Californian shelters (55%) [14]. Carriers (28%), particularly those with FHV or bronchiseptica infections, were at increased risk for onset of clinical URD. Only 5 cats were FHV-1 positive upon intake, however all developed URD. Similarly, of the 6 cats positive for B. bronchiseptica at intake, all but one developed URD. Although co-infections are common with B bronchiseptica, this bacterium alone is capable of inducing respiratory disease [31] which was the case in this study. In a UK study, 19% of cats with URD were positive for B. bronchiseptica alone [32]. However, an Italian study found more cases of co-infection (42 cases) compared to this bacterium alone (11 cases)[18]. In accordance with Bannasch and Foley [14], M.felis was the most prevalent pathogen but was not significantly implicated in the development of URD. Similarly, C. felis is a common pathogen isolated in cats with confirmed conjunctivitis [33]. However, it was not prevalent in this shelter. We concur with other authors [34] that this bacterium may not be an important risk factor for shelter cats. The H1N1 influenza virus can be transmitted from human to cats [35] or induced experimentally [36]. Although this study was conducted during the human H1N1 pandemic of 2009, no cases were identified nor have any cases been reported in other shelter studies to date. Overall, the only infectious agents with significant risk for onset of URD were FHV-1 and B. bronchiseptica. A recent study reported no significant difference in prevalence of URD between PCR positive and negative cats for these organisms [37]. These authors concluded that URD cannot be controlled alone by segregation of symptomatic animals due to a lack of strong correlation between subclinical infections and onset of URD. Rather they recommended similar biosecurity protocols and stress management practices for all cats. In addition to examination of carrier state as a risk factor, the multivariate analysis included age, gender, sterilization status, and source. Several authors agree that sex is not an important risk factor [14, 19] although one study identified adult females as a low risk group [5]. In our observations, there was no difference between male and female cats; however neutered cats were at greater risk for URD. Edwards et al. [20] found a similar trend, concluding that because most neutered cats were also owner-surrendered, they had likely not been exposed to URD pathogens and were therefore more vulnerable. In our study, more seniors than adults were neutered which may explain the association, in accordance with findings that age represents a significant risk factor for the development of clinical URD, with the very young and old being most vulnerable to disease [5, 15, 20]. In this study, age was not a significant factor. However inclusion in the study was restricted to cats older than 6 month and juveniles were poorly represented (n=50) which may account for the lack of significance. Although shelter staff received training to estimate the age of cats, exact age could not be determined for stray cats which may have introduced some bias. The finding that stray
cats were more susceptible to clinical URD than owner-surrendered cats agrees with other authors [38] and may have been influenced by the urban setting [16]. According to these authors, the high density of strays in urban settings increases the risk of contracting FCV and FHV-1 infections through social contact between cats (i.e., oral and nasal contact with secretions of shedding infected cats). The most significant risk factor for developing clinical URD in this study was time spent at the shelter. Most authors agree that the risk of developing clinical URD increases cumulatively with time spent in the shelter [5, 25]. However, one study reported a decline in clinical URD overtime, with most signs occurring within 50 days of admission [20]. It concluded that cats still at the shelter beyond 50 days were probably resistant to infection. Dinnage et al. [5] cautioned against such an interpretation because the number of cats remaining in studies usually decreases overtime, therefore the cumulative probability may be increasingly imprecise.

Overall the prevalence of subclinical infections and clinical URD were low in this shelter, which may be related to its policy of vaccination upon admission [39], placement of cats in quarantine upon admission [8] and good biosecurity [15, 30]. Although the practice is controversial, the immediate transfer of cats to an isolation ward upon onset of clinical signs is believed by some authors to reduce prevalence of disease [3, 20]. Others have suggested that the stress of moving to another cage may complicate disease [25] and increase severity [40, 41]. Finally, density of the population in a shelter is known to influence rate of transmission [42]. This shelter had a capacity of 120 cats with several separate housing areas which may have contributed to a lower rate of viral reactivation and transmission. Furthermore, 50% of the 250 cats had received a behavioural intervention to reduce emotional stress which was likely a contributing factor in the lower incidence of URD found in this shelter. Due to the variety of housing and animal care practices in various shelter the findings in this study cannot be extrapolated to other facilities.

Conclusion
This is one of the first studies performed in Canada to describe the epidemiology of infection status in cats upon admission to an animal shelter and risk factors associated with clinical URD overtime. With the exception of *Mycoplasma felis*, prevalence of subclinical infections and subsequent spread of pathogen was less than that observed in shelters from other countries. However, risk factors for URD, such as FHV-1 and *B. bronchiseptica* infections and stray status, were in accordance with risk found in other countries.
References


Chapter 7: General Discussion

During the last few decades, the interest in the link between emotions and health has been growing and has been examined across species using a wide range of methodologies. This is an area of investigation pertinent to the welfare of cats in animal shelters due to the shelter conditions which expose cats to both psychological and pathogenic stressors. For the most part, data supporting the emotion-health link in non-human species were obtained with invasive procedures. This study makes an important contribution to current knowledge with the examination of the emotion / health link with non-invasive measures. To date, few have differentiated among the underlying emotional states that may be affecting health in shelter cats. However, it has been demonstrated that different emotions (even when similarly valenced), have different effects on the cat immune system [1-3].

The attempt in this study to classify behaviours (usually labelled stress behaviours) according to their emotional quality may contribute to more specific application of interventions. Indeed, the first principle of effective intervention is to understand the underlying causes that may be contributing to a behaviour or health problem. The second is to develop interventions that target the underlying causes. For example, in an animal shelter, a cat motivated to hide because she is scared of people is likely to benefit from environmental enrichment that meets her need to appraise potential threats while remaining concealed. A cat frustrated by confinement is not likely to benefit from this type of intervention; rather she may benefit from some play time outside the cage. The interventions proposed in this study aimed to address the emotion-driven needs of cats for either safety, companionship, or stimulating activities as a complement to current enrichments provided to cats based on species-specific needs.

In this research, I aimed to answer the following questions: Firstly, can we identify the underlying emotional states of cats that motivate their behaviour? Secondly, what is the relationship between emotions, immunity and stress (HPA activation)? Thirdly, can we manipulate the emotions of cats to induce and sustain positive emotions? Finally, through the manipulation of emotions, can we enhance the immune system and reduce the incidence of URD?

Most commonly in the shelter cat literature, behaviours that deviate significantly from the normal activities expected of well-adjusted cats are labelled stress behaviours [4]. Because interventions targeted at the motivations underlying cat behaviour may better predict health outcomes than stress alone, I chose to label the groups of behaviours in accordance with constructs of the Motivational Theory of Emotions [5-7]. As an additional support for the choice of label, behaviours observed in
the study were compared to the vast literature which describes the responses of cats after stimulation of brain structures known to be associated with specific positive and negative emotions. Through these means, groups of behaviours were identified that classified cats as Anxious, Frustrated, Content and Apathetic (only uncovered in the second study).

In these studies, the experimenter (NG) assigning daily mood scores was aware of which cats were in the Control versus Treatment groups. A study in which the person coding the video would be blind to the assignment of cats to the groups would have been ideal but was not possible due to limited resources.

Anxious cats remained very still at the back of the cage, their bodies and ears flattened. Noise and people entering the room caused them to startle; and when approached they retreated further against the back wall of their cage. If moving to another location in the cage, they remained in a flat posture (crawling). They showed significant deviations from normal patterns of sleep, feeding, and grooming; all of which are essential for the maintenance of health. These behaviours, according to motivational theory, are elicited by the perception of threat and have evolved to facilitate the appraisal of stimuli that may affect safety.

Frustrated cats spent much of their time at the front of the cage. They meowed persistently, paced, pushed items around the cage, and spread litter and food around the cage. They appeared determined to find a way out of the cage by pushing on the door and the door latch, biting the cage bars or hanging on them, and pushing their paw through the bars. They paced and pawed at the walls and floor, and repeatedly scanned the cage looking for a way out. Although they were friendly and interactive, they occasionally and unpredictably (but not defensively) attempted to bite or scratch staff during the cleaning of the cage. These behaviours are thought to be elicited by a reduction in dopaminergic activity [8], likely motivated by thwarted access to desired resources or the inability to engage in rewarding behaviours.

Content cats sat calmly at the front of the cage watching activities or resting/sleeping in a relaxed position (lying on their side). They groomed, they ate, and they rubbed on objects in the cage. When walking, they were upright and moved confidently and calmly around the cage. When approached, they responded in a friendly manner. These behaviours have been associated with both the absence of threat (low arousal of the defence system) and the activation of the reward system. It is possible that for these cats, the access to food and a warm place to sleep were sufficiently rewarding.
Alternately, these cats may be bolder or have more experience with a wide range of environments, such that they were not concerned by the novelty of the situation.

Apathetic cats spent their day sleeping. They never groomed and ate only as a result of human intervention. These behaviours can be an indication of sickness [9], but may also result from a lack of control over the environment, repeated failure to attain a desired resource, or failure to avoid aversive conditions [10]. This group of behaviours was only identified in the second study and was, therefore, not included in the indices as they were not compared to other groups by PCA or examined together with cortisol.

The first study showed three outcomes related to behaviour and physiology: 1) Some Anxiety behaviours (flat, freeze and hiding) were negatively correlated with cortisol (although cortisol was significantly different only for freeze (when present or absent); and all Anxiety behaviours were negatively correlated with S-IgA (mucosal immunity). 2) Some Frustration behaviours (escape, pacing, pushing and aggression) were positively correlated with cortisol (significantly higher when behaviours were present versus absent); and all Frustration behaviours were negatively correlated with S-IgA. 3) Contentment behaviours (eating, walking and friendly to human) were positively correlated with cortisol; and all Contentment behaviours were strongly and positively correlated with S-IgA.

Despite the varying relationships between cortisol and specific behaviours, the mean cortisol value among the Anxious, Frustrated and Content cats was not significantly different. This may have been due to the limited number of behaviours correlated to cortisol (either negatively or positively), or to the small sample size (n=34). Regardless, these results have important implications for hypothesis generation; such as the hypothesis that hiding may be an important behaviour for regulating HP axis activation [11]. Another notable finding was that cortisol and S-IgA were not correlated. This may also be explained by the small sample size. However, many studies have shown that even when adrenals are removed, animals exposed to psychological stressors can experience immunosuppression similar to that induced by sustained HPA activation [12]. The most notable finding was the strong relationship between a positive emotional state (Contentment) and S-IgA. Anxious and Frustrated cats had significantly lower S-IgA concentrations than did Content cats. Further, in the second study, the clinical implications became evident. Content cats were significantly less likely to develop URD than Anxious and Frustrated cats. According to Broom [10], any condition that affects welfare negatively has consequences for pathology making disease
more likely to occur and more debilitating when it occurs than in animals with good welfare. These results highlight the complexity of the relationships among emotions, stress, and the immune response.

Dawkins [13] asserts that giving animals what they need and what they want is the best way to ensure good welfare. The striking contrast between the behaviours of Anxious and Frustrated cats provides clear indication as to their needs and desires when examined in the motivational framework. According to this framework, defensive and hyper vigilant behaviour indicate the perception of danger and a need to appraise potential risks. Conversely, repetitive and destructive behaviours indicate frustration due to the thwarted access to desired activities or resources (e.g., access to outdoors, food, human contact). A common recommendation given by shelter professionals and scientists (including myself) is to provide all shelter cats with a structure in which they can hide [14-16]. Whilst this certainly facilitates the task of appraising potential dangers while remaining concealed, thus contributing to a feeling of safety, it does not address the underlying perception of danger. The gentling treatment proposed in this study aimed to address the root cause of anxiety by changing the perception of some of the ambivalent cues that elicit anxiety in the first place – the unfamiliar human. Ideally, both the environmental enrichment (the box) and the gentling would be provided together. However, our findings show that even in the absence of environmental enrichment, gentling can induce contentment and reduce URD. According to motivational theory, contentment is a state indicative of low arousal of the defence system. Although, this hypothesis cannot be directly tested, these results suggest that gentling may have changed the perception of danger, thus removing the need to hide. There may, however, be competing hypotheses.

The Frustrated cats gave clear behavioural indicators demonstrating their desire to get out of the cage. Opening the door to the cage of a cat that is pushing on it or meowing persistently to attract attention is quite effective in instantly reducing emotional arousal. Acquiring a desired resource (reward) activates the dopaminergic neural system which releases dopamine, known to induce pleasure [17, 18]. The component of the treatment which involved learning a new task and which gave frustrated cats control over an appetitive reward (at least during the treatment) may have had this effect. Although, changes in biochemistry were not measured in this study, the behaviours observed during the training sessions (purring, rubbing, tail up, chirps) may be indicators of pleasure. Further research may elucidate this hypothesis. It has been proposed that removing enrichment may cause or increase frustration. Mice provided with enrichment which is subsequently removed engage in more frustration-related behaviours than mice that were never
given enrichment [19]. It is possible that, for some cats, time out of the cage and temporary control over an appetitive reward may subsequently cause an increase in frustration upon return to their cage. Although this was not observed in this study, further research may be needed to examine other treatments that may reduce the frustration of cats while in the cage. For example, the use of food puzzles has been proposed as enrichment suitable for the cage environment [14]. Nonetheless, these findings clearly indicate the risks associated with failing to provide interventions. Anxious and Frustrated cats that did not receive treatments experienced negative affect longer (thus prolonging suffering) and had a significantly higher prevalence of URD than did those that received treatments.

While it is, of course, important to reduce the negative emotions of shelter cats, it is equally important to help cats sustain a positive emotional state once they adapt to the shelter or if they were content upon admission. The Content cats that did not receive treatment (positive human interaction) were significantly more likely to develop Frustration over the course of the study and had a higher prevalence of URD. Even when cats remained Content, those receiving treatments had higher S-IgA than non-treated cats. A meta-analysis of studies reported that social support (defined as the feeling of being cared for and loved) decreases susceptibility to various ailments and enhances health in humans [20]. Resources in shelters may be limited, and volunteers may, by necessity, focus their attention on cats most visibly in need. Spending time with those that are coping more effectively with the shelter environment may, in the long run, reduce respiratory disease and the cost of caring for sick cats.

An important goal of this research was to examine the link between emotion and health using non-invasive methods. The research contributes to the understanding of mucosal immunity and the effect of emotions on intestinal IgA. It has been proposed that the wet weight of a faecal sample is dependent on the water content and that it is impossible to measure the yield of S-IgA due to water content variation [21]. Our findings show that, despite the potential temporal- and diet-related variability, S-IgA concentrations were consistent overtime in individuals and varied greatly according to emotional state. This non-invasive measure of S-IgA, as a measure of wellbeing and susceptibility to respiratory disease, is of clinical and ethical importance. There is, however, a need to standardize the assays and to normalize reported values. This study identified additional challenges that may stimulate further research. The time course between a stressful event (natural or ACTH challenge-induced) and the appearance of cortisol metabolites in faeces is well documented [22, 23]. Conversely, there is an important gap in knowledge regarding a similar time course for intestinal IgA in response to psychological or antigen challenges. Much research has examined the
efficacy of intranasal vaccination [24-26], which aims to increase IgA secretion to mucosal sites. To my knowledge, the baseline range for IgA in healthy, well-adjusted felines is not known. Despite many unknown factors, the documented importance of the protection afforded by S-IgA against URD pathogens makes this an important area of investigation. It is also well documented that cell-mediated immunity can be manipulated with behavioural interventions. The notion that emotions can easily be manipulated to improve immune function is of clinical and practical importance [27-29]. In light of the importance of mucosal immunity for the health of shelter cats, behavioural manipulation able to alter the immune response of cats is an important topic of investigation.

Interventions in this study that resulted in positive emotional manipulation of the cats stimulated secretion of intestinal IgA. In addition, cats that became Content in the non-gentled group had lower S-IgA than the Content cats from the gentled group. Similar findings were reported in anxious humans receiving a back rub. Both massaged and non-massaged people experienced a reduction in anxiety scores in response to spending 10 minutes relaxing in a comfortable and quiet area. However, only the group receiving the massage experience an increase in IgA [30]. A gentle touch may have a stimulating effect on S-IgA, mediated by mechanisms other than emotion. Indeed various types of tactile interventions not specifically designed to alter emotions have been shown to stimulate S-IgA [31, 32]. The skin has many receptors which, when touched, may stimulate the mesolimbic system and activate secretion of health-enhancing biochemical [33, 34].

In addition to furthering knowledge on the relationships among emotions, stress, immunity and health in shelter cats, and the utility of interventions informed by the Motivational Theory of Emotions; this research makes an important contribution to methods for the interpretation of multivariate behavioural data. PCA is commonly used in behavioural studies [35] and interpretation of results based on component loadings is usually favoured over graphical examination of the PCA biplots. Although, the table of loadings which resulted from this analysis was examined (data not included), it was clear that the biplot provided much more information about the structure of the data within and between variables in the PCA subspaces. This finding suggests the worth of comparative study of the two approaches for interpreting PCA when applied to behavioural data.

An important issue which is commonly overlooked when relying on tables of loading is that the cosine value associated with each loading indicates how important it is to the component. It is difficult to verify whether results are well interpreted. For example, importance may be attributed to a loading value (>0.4), even in cases when the cosine value was low. This is particularly true when software does not report the cosine (and many do not), or if cosines are not examined. The use of cosines can reduce the risk of wrongly assigning meaning to a relationship between two variables.
In the biplot, the cosine value is evident (either visually or by measuring the angle between vectors) and misinterpretation is less likely. In this study, PCA showed a correlated group of behaviours that we labelled Frustration, but it was only through the measurement of the vector angles that 2 subgroups emerged. Although related to each other by their shared proximity to some behaviours, there was a qualitative difference between the subgroups that had important ethological value. The cosine of the vectors indicated that aggression and meowing were two behaviours that differentiated cats within the Frustration group. Cats that engaged in persistent meowing were not likely to express redirected aggression and vice-versa. This is an important finding which may have been difficult to discern according to the variable loadings alone. Similarly to research showing that the two types of responses to threat (defensive attack defensive retreat) have different effects on immune response [2, 3], further research may show similar differences according to the two types of Frustration responses.

Contrasts between groups of behaviours are also easier to detect in a biplot. For example, it was easy to identify that Contentment behaviours were on a continuum with Anxiety, because they were in opposite quadrants (likely opposites of the same motivation system). As a caution, rotation of factors in PCA is usually necessary to simplify the interpretation of results (assigning bigger values to big numbers and smaller values to small numbers). However, orthogonal rotations (depending on the data) can also contribute to over or under-representation of relationships between variables. Biplot methodology does not require the use of orthogonal rotations as the relationships are examined by visual inspection of coordinates and do not need to be simplified for interpretation.

The use of biplot methodology in animal behaviour studies is uncommon. One author who applied it to heart rate variability in farm animals reported that geometric analysis of data provided more detailed information about the data than conventional PCA analysis [36]. I hope that this research offers incentive to further test this approach in animal studies.

The most important application of this research is in the identification of the emotions of shelter cats. Although the treatments applied in this study were effective, it is unlikely that they would be if they were not applied to the specific problem they were designed to address. The Indices of Cat Wellbeing constructed in this study contain a limited number of behaviours and can quickly identify the emotions of cats. However, they are the ones that also provide information about susceptibility to disease (based on correlated S-IgA and the likelihood of developing URD. Further research may uncover more behaviours that are specifically linked to immunity and health in shelter cats.

Having myself worked in animal shelters for over a decade, I have witnessed firsthand the creativity of shelter staff in devising various types of enrichments. Armed with a better understanding of the underlying emotional states of cats, shelter workers can better identify appropriate applications of
the enrichments they wish to provide. Currently, a major focus in shelters is to reduce stress. This need has also been raised in epidemiological studies of URD [37-39]; contributing to the awareness of the important implications of psychological factors in respiratory disease, and to the welfare of cats. However, reliance on concepts of stress may also result in a “one size fits all” strategy for addressing poor welfare; helping some cats, but leaving others untreated. We can imagine a scenario where a frustrated cat is provided with non-specific enrichment (e.g., the commonly used hiding box), leaving the source of frustration unaddressed. In this study, some of the cats became apathetic after several days of non-treated frustration - a common outcome of chronic frustration [40, 41]. Hypersonmia, together with inhibition of feeding and drinking may result in dehydration putting the apathetic cat at higher risk for URD and even mortality, particularly if she is sleeping in the box and goes unnoticed. Application of an appropriate intervention may reduce the likelihood of frustrated cats becoming apathetic; with the potential for improving health outcome.

The gentling treatment tested in this study may have important shelter application. In the gentling study, some of the Anxious cats became fearful when I approached the cage and attempted to touch them. They were defensive and aggressive (displaying flattened ears, pupil dilation, increased muscle tension, growling, hissing, lip licking and striking). These cats were gentled with an extendable stick equipped with a rubber tip (called a target stick and normally used for clicker training). By day six, none of the cats gentled in this way were aggressive; whereas non-gentled cats that were similarly fearful/aggressive were still aggressive by the end of the study. In North American shelters (and probably worldwide), staff are called upon to determine the adoptability of cats. This is a difficult and emotionally challenging task, as aggressive cats (particularly if classified as feral) are routinely euthanized following a legal holding period of (usually) 3 or 4 days [42]. According to these protocols, the 25 cats in this study that became fearful / aggressive when approached would have been euthanized. In shelters where euthanasia is not practiced, these cats are less likely to receive positive attention from staff and volunteers, are less likely to be selected by an adopter [16], and are at higher risk for URD. The use of mechanical gentling may be useful for reducing the incidence of cat bites, reducing euthanasia based on the aggression criterion, and for improving the welfare of the most vulnerable cats (the fearful ones).

Conclusion

Cats in animal shelters experience a range of emotions such as Anxiety, Fear, Frustration, Apathy and Contentment. Behavioural treatments used in this study successfully induced and helped sustain
positive affect (Contentment). Moreover, treated cats had enhanced IgA-mediated immunity and lower incidence of URD.

References


Appendix: Suggestions for application of research findings in animal shelters

Introduction

The University Of Queensland Indices of Cat Wellbeing (UQ-ICW) is a behavioural tool for the assessment of emotional states in shelter cats. It was developed by comparing groups of behaviours to physiological measures of stress (cortisol) and mucosal immunity [immunoglobulin A (S-IgA)]. Thus the behaviours included in UQ-ICW are those that are now known to be significantly related to changes in immunity. S-IgA is an essential antibody of local immunity (in nose, mouth, eyes, intestinal track). It prevents pathogens that cause URD from entering the body and infecting the cat. The higher the concentration of S-IgA on mucosal sites, the less a cat is likely to develop URD due to pathogens that were inhaled or ingested.

Several behaviours were added to UQ-ICW based on findings in subsequent studies. Although, these behaviours were not individually validated with S-IgA, they were found to occur exclusively and frequently with others in their emotional category and contributed to overall changes in S-IgA. Further a category labelled Apathy was added based on behaviours observed in some of the Control cats (not receiving treatment) that had been rated as Frustrated for several days. The behaviours were: loss of interest in feeding, grooming and interaction with humans together with hypersomnia. Apathy was not included as an emotion as it was not validated during the construction of the UQ-ICW.

Risk factors for poor wellbeing (negative emotions and high susceptibility to URD)

Cats were found to be at risk for URD as soon as they entered the shelter (due to inhibition of S-IgA production). Thus it is recommended that emotional states be assessed upon admission and that cats be provided with a treatment appropriate for their emotional state as soon as possible.

Findings from this study indicated that:

- Senior, owned, spayed females are at higher risk for anxiety
- Stray, intact males are at higher risk for frustration
- Juvenile, females are most likely to be content
- Anxious cats and frustrated cats are at higher risk for URD (due to low immune protection)
- Content cats are at lower risk for URD.

Assessing cats

* In this study, cats were observed by video. The responses of cats may be affected by the presence of a human. Hopefully, future research will examine the different behavioural responses to observation done by a human compared to those done by camera. With current technology, it may be possible to install webcams in cat rooms that may facilitate the use of UQ-ICW in shelters.
Appendix

*Intake assessment*

In this study, cats were assessed upon admission based on a 1 hour observation by video followed by an approach test during which the assessor opened the cage and attempted to touch the cat. In practice, it may not be possible to observe cats for that long. However, with the exception of a few cats, most were found to maintain the same emotional response throughout the first hour of observation. Thus observing the cat for a few minutes following placement in the cage and at the end of an hour may provide an appropriate rating in addition to responses to being approached and touched (although this has not been validated).

*Daily assessment*

In this study, cats were observed by video 5-10 minutes each hour per day. Anxiety and Contentment were found to be chronic states (lasted more than 80% of observation time per day). Thus observing cats for several minutes a few times per day may provide sufficient indicators to determine if cats are Anxious or Content (although this has not been validated).

Frustration occurred in bouts and was observed more than 10% of awake time. In practice, Frustration may be more difficult to assess as it occurs in bouts and more often in the absence of humans. However, there were environmental indicators for Frustration such as spilled food, water and litter and items moved around in the cage. These were evident within minutes of the cage being cleaned. If a cat is suspected of being frustrated, a person may be assigned to watch the cat (from afar) for an hour or more.

*Interventions to reduce level of risk for URD.*

In this study, Control cats were at higher risk for URD than cats that received treatments even when these cats were assessed as Content.

Treatments should be provided four times per day for 10 minutes (different amount of treatment may be effective but were tested in this study).

*Treatment for Anxious cats*

Gentling is a type of gentle petting restricted to the head and neck area and provided in short repeated strokes (stop/start lasting less than one minute each). Gentling was effective in increasing S-IgA and reduced incidence of URD.

Some cats, particularly those that are Anxious may become fearful when touched. When cats show defensive aggression (hissing, growling, attack) when approached or touched, it is necessary to use...
a tool to provide the Gentling (See Chapter 3 for details). Once the cats relax in response to Gentling with the tool, gentling by hand can be provided.

_Treatment for Frustrated cats_

Cognitive enrichment should be provided to Frustrated cats. It involves taking the cat out of the cage for a 10 min training session. In this study, the cats were trained to perform a behaviour called “high five” (cat’s paw touches the experimenter’s hand). The training involved shaping the high five by breaking the desired behaviour into steps. Each step such as approach, sit, paw lift and touch experimenter’s hand is reinforced and rewarded while all other behaviours are ignored. The correct behaviours are with an auditory cue (with the clicker) that indicates to the cat that a reward is coming (e.g. food, petting or access to a toy for a few seconds).

*Prior to shaping a behaviour using an auditory cue, the first few sessions are used (only) to associate the click sound with the reward.*

_Treatment for Content cats_

The goal of providing positive human interaction to Content cats is to help them sustain Contentment. In this study, cats that did not receive treatments were at higher risk of developing frustration and at higher risk for URD.

Positive human interaction involves spending time with a cat engaged in an activity they seem to enjoy. In this study, cats were offered a brush to rub against, a toy to play with or petting (without restriction to any specific area of the body).

_Pictorial Ethogram_

The following pages illustrate each behaviour included in UQ-ICW and the treatments provided according to emotional classification.

*Although, shelter cats engage in a wide range of behaviours, the ones included in UQ-ICW were those found to be significantly correlated to S-IgA and therefore are relevant to the assessment of well-being (mental health and susceptibility to URD).*
Table 1: Anxiety behaviours included in UQ-ICW that were significantly correlated to S-IgA

<table>
<thead>
<tr>
<th>Anxiety behaviours observed &gt; 80 % of observation time</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flat</strong></td>
</tr>
<tr>
<td><strong>Freeze</strong></td>
</tr>
<tr>
<td><strong>Hide</strong></td>
</tr>
<tr>
<td><strong>Startle</strong></td>
</tr>
<tr>
<td><strong>Crawl</strong></td>
</tr>
<tr>
<td><strong>Retreat</strong></td>
</tr>
<tr>
<td><strong>Self-maintenance</strong></td>
</tr>
<tr>
<td><strong>Absent</strong></td>
</tr>
</tbody>
</table>
Fig. 1 Hiding behind litter in flat posture.

Fig. 2 Hiding under towel.
Fig. 3 Hiding under litter tray.

Fig. 4: Flat postures.
Fig. 5: Defensive retreat from human (no aggression)
Fig. 6 Positive response to approach (a) and touch test (b) indicating that gentling by hand is appropriate.
Fig. 7: Pre, during and post treatment. (a) Anxious cat (hiding under towel) responds positively to gentling, b) extends paws, rubs on hand and c) approaches front of cage (still under towel) post treatment.
Fig. 8: Pre gentling (flat posture), during (relaxes extends paws) and post (reduction in flattened posture)
Fig. 9: Head posture rated as positive response to gentling (top the cat rubs on hand, bottom cat raises chin)
Fig. 10: Defensive attack response (fear) to the Approach test indicating the need to gentle using a tool in response to an attempt to gentle by hand.
Fig 11. Positive response behaviours and head posture to gentling with a stick for a cat showing defensive attack (fear) upon approach.
Table 2: Frustration behaviours included in UQ-ICW that were significantly correlated to S-IgA.

<table>
<thead>
<tr>
<th>Frustration behaviours observed &gt;10% of awake time</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meow</strong></td>
</tr>
<tr>
<td>Persistent meow not related to anticipation of food</td>
</tr>
<tr>
<td><strong>Escape bouts</strong></td>
</tr>
<tr>
<td>Very active and includes one or more behaviours performed persistently or frequent in repeated bouts and occurs mainly at the front of the cage: On hind limbs, pawing wall/ floor, paw through door, push on door or latch, hanging on cage door with body inverted</td>
</tr>
<tr>
<td><strong>Scan</strong></td>
</tr>
<tr>
<td>Visual scanning of the cage</td>
</tr>
<tr>
<td><strong>Push</strong></td>
</tr>
<tr>
<td>Hits or throws objects around the cage in a destructive manner using head, body or paws (not related to play.) Spills food bowls, and litter. May push on cage door with head, paws or body.</td>
</tr>
<tr>
<td><strong>Pace</strong></td>
</tr>
<tr>
<td>Repetitive and fast walking back and forth in the cage particularly by the door</td>
</tr>
<tr>
<td><strong>Redirected aggress.</strong></td>
</tr>
<tr>
<td>Quick bite, swipe or scratch during interaction with human without defensive vocalizations, body posture or retreat. May occur in bouts during otherwise friendly interactions</td>
</tr>
<tr>
<td><strong>Bar biting</strong></td>
</tr>
<tr>
<td>Bites or lick cage bars persistently or in repeated bouts</td>
</tr>
<tr>
<td><strong>Absent</strong></td>
</tr>
<tr>
<td>All Anxious behaviours and lie on side</td>
</tr>
</tbody>
</table>

*bar biting was not included in the UQ-ICW (not validated with cortisol or S-IgA) but added as it was frequently observed together with other Frustration behaviours in subsequent studies.*
Fig. 12: Stand or sit persistent meow
Fig. 13 Scanning
Fig. 14: Hanging on door (escape.bouts category)
Fig. 15a: Upside down (1) (escape.bouts category)

Fig. 15b: Upside down (2) (escape.bouts category)
Fig.16: Bar biting or licking (additional behaviour found in Frustration study and included in UQ-ICW but not validated with physiological measures.
Fig. 17: Pushing items around the cage, pushing on door and paw through door.
Fig. 18: On hind limbs with attempt to open door latch (from escape.bouts category)

Fig. 19 Pawing at wall (escape.bouts category)
Fig. 20: Paw through door (escape.bout category)
Fig. 21: Cognitive enrichment (training session to teach high five behaviour). 1) Rewarding approach. 2) approximation of sit 3) and full sit. 4) Rewarding touch with paw (even though cat is no longer in sit posture).
Fig. 22: Shaping high five behaviour with clicker stick (when working with cats that need additional luring).
Table 3: Contentment behaviours included in UQ-ICW that were significantly correlated to S-IgA.

<table>
<thead>
<tr>
<th>Contentment behaviours &gt; 80% of observation time</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sleep/rest</strong></td>
<td>Relax body posture and neck, eyes closed or semi closed</td>
</tr>
<tr>
<td><strong>Lie on side</strong></td>
<td>Laterally recumbent, body stretched, neck and ventral area exposed, limbs and tail extended</td>
</tr>
<tr>
<td><strong>Front.sit</strong></td>
<td>Sits at the front of the cage, calmly looking around</td>
</tr>
<tr>
<td><strong>Groom</strong></td>
<td>Licks fur or paws and rubs head with paws (without chewing or pulling coat)</td>
</tr>
<tr>
<td><strong>Walk</strong></td>
<td>High body posture, normal gait and pace to move around the cage (not ongoing or repetitive)</td>
</tr>
<tr>
<td><strong>Eat / drink</strong></td>
<td>Takes food or water into mouth and ingest. Particularly in the presence of humans. Does not spill food or water around the cage</td>
</tr>
<tr>
<td><strong>Friendly</strong></td>
<td>Approaches front of cage or human in amicable manner.</td>
</tr>
<tr>
<td><strong>Absent</strong></td>
<td>All Anxiety behaviour and persistent meowing, scanning, repetitive or escape behaviour</td>
</tr>
</tbody>
</table>
Fig.23: Sleep and lie on side. Behaviour correlated with highest S-IgA

Fig.24: Resting and lie on side
Fig. 25 Sitting and calmly watching activities (labelled sit.front)

Fig. 26: Eating
Fig. 27: Grooming

Fig. 28: Rubbing was a contentment behaviour but was not very well or exclusively represented in that dimension (it also occurred in frustrated cats.)
Fig. 30: Friendly response to human (walks forward, sniffs, rub).
Fig. 31: Positive human interaction treatment (petting, brushing, playing). This cat responded positively to petting and brushing but was not interested in playing.
Fig. 32: Behaviours (crouch and ventral high) commonly observed but not well represented in their respective emotional dimension meaning that they could occur other emotional dimensions.