



Associations of behaviour with secretory immunoglobulin A and cortisol in domestic cats during their first week in an animal shelter

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ABSTRACT

We tested the hypothesis that during their first week in an animal shelter, cats exhibit groups of behaviours that are connected to mucosal immune and adrenal responses. The behaviour of 34 cats was observed from admission to day 5 and immunoglobulin A (S-IgA) and cortisol were quantified from faeces. A multidimensional model constructed by Principal Component Analysis indicated the presence of three distinct behavioural dimensions. Behaviours forming dimension 1 were hiding, flat postures, freeze, startle, crawl and retreat from humans. These were significantly contrasted ($R = -0.6$ to -0.4) to dimension 3 behaviours which included normal patterns of feeding, grooming, sleeping and locomotion, sitting at the front of the cage while calmly observing activities, sleeping or resting while lying on their side, rubbing on cage items and friendly behaviour towards humans. Dimension 2 behaviours included persistent meowing, scanning, pacing and pushing, together with bouts of destructive behaviour, attempts to escape and redirected aggression. Dimension 2 was not significantly contrasted to dimension 3 ($R < -0.4$ except for sleep = 0.6) or dimension 1 ($R \leq -0.2$). S-IgA values were greater ($P < 0.001$) for cats clustered in dimension 3 (mean $7.1 \pm 0.5 \log_e \mu\text{g/g}$), compared to dimensions 1 and 2 which were not significantly different ((1) 5.6 ± 0.6 ; (2) $5.6 \pm 0.7 \log_e \mu\text{g/g}$). Cortisol values were similar for the three dimensions. Despite the difficulty in generalising the results to the shelter cat population due to small sample size, our findings suggest that behaviour is a good indicator of mucosal immune function in shelter cats. This may be of clinical significance for the management of upper respiratory disease in animal shelters.

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1. Introduction

Upper respiratory infections (URI) are pervasive in shelter cats worldwide, and represent the most common

health problem that results in euthanasia of both cats and kittens (Dinnage et al., 2009). Entering an animal shelter represents a major change in routine and exposes cats to known stressors such as confinement, exposure to unfamiliar humans, animals and overcrowding. These conditions increase their susceptibility to respiratory pathogens (Pedersen et al., 2004) that are found in high concentrations in multi cat environments (Hurley, 2005). In humans, distress increases the likelihood of contracting respiratory infection and lengthens recovery time (Cohen, 2005; Corbett et al., 2010). Scientists have

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argued that emotions compromising the health of animals in shelters must be identified and addressed (McMillan, 2002; Griffin, 2012). Several studies have shown that electrostimulation targeted at areas of the brain that elicit emotional responses in cats, such as anxiety, fear and stereotypic restlessness also alter physiological correlates, particularly cell-mediated immunity (Kojima et al., 2000; Mori et al., 2001; Kaname et al., 2002; Sumida et al., 2004).

Many of these studies utilised invasive neural stimulation, but non-invasive measures of affective states that relate to behaviour and immunity of cats following entry to an animal shelters are now needed. More specifically, and because it can be measured non-invasively, mucosal immunity, the first line of defence against upper respiratory infections (URI), warrants investigation as to how it is affected by the entry of cats into animal shelters.

Secretory immunoglobulin A (S-IgA), the major class of mucosal antibody, prevents respiratory pathogens that are inhaled or ingested from penetrating the epithelial walls at mucosal sites (Hannant, 2002; Stokes and Waly, 2006). Feline S-IgA has been quantified in saliva (Harley et al., 1998) and faeces (Yamada et al., 1984; Rodriguez et al., 2007), however the influence of affective states, inferred from behavioural responses, on secretory activity has not been examined. The effect of presumed affective states and psychological stress on S-IgA has been examined in some other species, including sheep, rats and mice (Napolitano et al., 1995; Eriksson et al., 2004; Rammal et al., 2010), but the feline mucosal immune system bears a stronger resemblance to that of pigs, dogs and humans (Stokes and Waly, 2006). In the latter species, acute and chronic stressors appear to increase and inhibit S-IgA secretion, respectively. A series of environmental and social stressors have been shown to inhibit S-IgA secretion in several species (pigs: Royo et al., 2005; dogs: Berteselli et al., 2005), although brief negative experiences may increase secretion (Muneta et al., 2010). Reduced secretion may be reversed by providing positive environmental and social interactions (e.g. Taniguchi et al., 2007; Noto et al., 2010).

Despite apparently confounding results due probably to variation in S-IgA levels across faecal (Carlsson et al., 2007) and salivary (Harley et al., 2003) samples, as well as fluctuation in the circadian rhythm (Kikkawa et al., 2005), affective states appear to be significant moderators of S-IgA. However, non-invasive measure of affect remains elusive in non-human animals. Nonetheless, it is generally accepted that affect can be inferred from behaviour (e.g. Reefmann et al., 2009; Mendl et al., 2010). Moreover, in cats, behavioural expressions of emotions elicited by hypothalamic or pharmacological stimulation are undistinguishable from the behavioural responses observed in response to threats, such as presentation of a dog, restraint, waving a stick and poking them (e.g. Brudzynski et al., 1990).

The objectives of this study were (1) to identify how the short-term behavioural expression of cats entering a shelter may allow them to be clustered into emotional profiles, and (2) to explore how such profiles may relate to cortisol and S-IgA secretion.

2. Materials and methods

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

2.1. Animals

Forty cats entering 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. Of these, 53% ($n = 18$) and 47% ($n = 16$) were designated by the relinquisher as strays and owned, respectively, and this classification was checked by the receiver. No cats were rejected and of the 40 cats, 34 remained to the end of the study. Twenty-two were adults (1 to 7 years of age), 7 seniors (>7 years of age) and 5 juveniles (<12 months); 8/18 females and 9/16 males were sexually intact during the study. All cats were examined at intake by a Veterinary Health Technician. Vaccination was delayed until after the study (day 7); however, cats were dewormed before starting (Strongid® T. Pfizer, Kirkland, Québec, Canada).

2.2. Housing

All cats were housed individually in a stainless steel cage ($76 \times 76 \times 71$ cm) furnished with a litter box and non-absorbent cat litter (Veterinary Concepts, Spring Valley, Wisconsin, USA). Stainless steel food and water bowls were fastened to the cage door, and a towel provided for bedding. Cages were either barren or equipped with a Hide, Perch & Go Box™ (Animal Behaviour Systems Australia Pty Ltd, Hoppers Crossing, Victoria, Australia) (Gourkow, 2007) of dimensions $46 \times 41 \times 31$ cm, to simulate housing provided in different shelters (Kry and Casey, 2007). Cats were randomly allocated to cages with and without a box, however the final ratio (20:14, respectively) was unequal because some cats from the 'no box' group were removed from the study after 4 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 $20 \pm 2^\circ\text{C}$ was maintained. Feed was provided twice daily (07:00 h and 17:00 h) (Science Diet, Hill's Pet Nutrition, Inc. ®/™, Vancouver, British Columbia, Canada) and fresh water was provided ad libitum.

2.3. Behavioural observations

Outside each cage, an infrared camera (Sony CCD25 M crystal-View Super Hi-Res ICR IR Camera SLED w/9–22 mm Vari-focal Lens, Microtech Advanced Technologies Ltd, Vancouver, British Columbia, Canada) was mounted at cage height on a rod suspended from the ceiling at 1 m from the cage door. Footage was both viewed in real-time in an adjacent room and stored for subsequent analysis. The starting point was an ethogram limited to 40 behaviours reported in observational studies of caged and household cats (Fangel and Kaada, 1960; Ursin, 1964; Kessler and Turner, 1997; Bernstein, 2006). To facilitate observations, the behaviours

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