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Effect of interactions with humans on behaviour, mucosal immunity and upper respiratory disease of shelter cats rated as contented on arrival

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ABSTRACT

Sustained positive affect may decrease vulnerability to upper respiratory infections in cats admitted to a shelter. Incidence of upper respiratory infections was examined in cats rated as Content upon admission to an animal shelter when provided with or without treatment to sustain contentment. Ninety-six cats rated as Content upon admission were provided with either human interaction, including petting, playing, and grooming, in four 10 min sessions/d for 10 days or were exposed to a control treatment of a human standing in front of the cage with eyes averted for the same period. Changes in emotional state and mucosal immune responses were measured daily in treated and control groups. 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 herpes virus type 1, feline calicivirus, *Mycoplasma felis*, *Chlamydophila felis*, and *Bordetella bronchiseptica*. The onset of upper respiratory disease (URD) was determined by veterinary staff based on clinical signs, including ocular or nasal discharge. Treated cats were more likely to remain Content (Incident Rate Ratio [IRR]:1.13, Confidence Interval: 0.98-1.30, P <0.0001) and less likely to be rated as Anxious or Frustrated than Control cats over a 10 day period (IRR: 0.61, 95% CI: 0.42-0.88, P =0.007). Feline secretory IgA (S-IgA) quantified in faeces by ELISA techniques, was greater for Treated than Control cats (1451 Vs 846 μg/g). Within the Treatment group, S-IgA was greater for cats that sustained Contentment throughout the study period compared to cats that became Anxious or Frustrated (1846 Vs 1394 μg/g). An increasing proportion of Control than Treated cats shed pathogens over time (Control 22%, 36%, 61%; Treated 35%, 26%, 32% on d 1, 4 and 10, respectively; P =0.006). Control cats were more likely to develop URD than Treated cats (HR 2.9, CI: 1.30-6.67, P =0.01). Cats that responded positively to Treatment had a lower incidence of URD than negative responders (P =0.02). We conclude that the provision of human interaction treatments to shelter cats can facilitate sustained contentment, enhance secretion of S-IgA, and reduce incidence of URD.

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1. Introduction

Humans with a tendency to be happy, pleased, relaxed, and lively are less susceptible to upper respiratory infection when compared to those with less happy dispositions (Cohen et al., 2003), and exposure to pleasant stimuli that induce positive affect stimulates mucosal immune response (Watanuki and Kim, 2005). Research with cats suggests a mechanism and cross species validity, since cats that are Content even in stressful situations show a higher level of secretory immunoglobulin A (s-IgA) than those that are Anxious or Frustrated (Gourkow, 2014a), and treatments that induce contentment also stimulate s-IgA (Gourkow et al., 2014b). This mucosal immune antibody is the first line of defence against pathogens causing upper respiratory tract disease URD (Adédoyin et al., 2007), which are found in high concentrations on shelter equipment, hands and clothing of caretakers (Hurley, 2011). The glue-like substance in the mucosae of the nose, mouth and intestinal tract prevents pathogens from penetrating the epithelial wall (Brandtzaeg, 2003). Conversely, IgA deficiency is characterized by recurrent signs of (URD) in adult humans (Jacob et al., 2008), children (Sandin et al., 2011) and dogs (Felsburg et al., 1985).

Similar to cell-mediated immunity (Mori et al., 2001), the mucosal immune system is affected by emotional states and can be manipulated by behavioural modifications (Ader and Cohen, 1975; Rogers et al., 1976; Wayner et al., 1978). Relaxation therapy stimulates secretion of S-IgA (Taniguchi et al., 2007) and lowers

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incidence of URD (Cohen, 2005; Hewson-Bower and Drummond, 1996; Hucklebridge et al., 2000; Segerstorm and Miller, 2004 Taniguchi, 2007). The growing awareness of the importance of positive affect on respiratory health in shelter cats is reflected in current guidelines and standards for the care of animals in shelters (Newbury et al., 2011). These authors proposed that enhancing emotional wellbeing should hold the same significance as other aspects of animal care, such as nutrition and veterinary care. In humans, social support (positive human interactions) facilitates coping during times of stress and decreases susceptibility to disease (Wang et al., 2003). Even looking at a photograph of a favourite person increases IgA concentration (Matsunaga et al., 2008). In shelter dogs, positive human interactions, including petting, grooming, and walking with a familiar person, reduces cortisol and heart rate (Bergamasco et al., 2010; Coppola et al., 2005; Hennessy et al., 1998). Cats may similarly benefit from such interactions. For example, laboratory cats prefer interactions with humans to playing with toys (De Luca et al., 1992).

The hypothesis that sustained positive affect may be achieved in shelter cats by providing positive human interactions was examined, together with its effects on S-IgA and incidence of upper respiratory infection.

2. Material and methods

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

2.1. The shelter and experimental ward

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.

A housing unit located on the second floor of the shelter was used as the experimental ward. This room was maintained at a constant temperature of 20 ± 2 °C, and was naturally lit with the provision of artificial light 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, the experimenter and an intern (volunteer research assistant). In common with most shelter environments, some sounds of dogs barking, as well as people walking and talking nearby, were audible to the human ear. The experimental ward included a food preparation area out of sight of the cats. Feed was provided twice daily at 0700 and 1700 h and comprised 70 g of age-appropriate pellets and approximately 30 g of wet food (Science Diet, Hill's Pet Nutrition, Inc. ®/Ô Topeka, KS, U.S.A.). Fresh water was provided ad libitum. Feeding was undertaken by the intern, the experimenter or shelter staff.

The cat housing in the experimental ward consisted of 20 stainless steel cages (76 × 76 × 71 cm). Each was furnished with litter boxes and non-absorbent cat litter (Veterinary Concepts, Wisconsin, U.S.A.), a stainless steel food and water bowl, and a towel for bedding. Each cage was fitted with 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, Canada) 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

Shelter staff cleaned cages daily by removing all waste, changing bedding, and wiping walls with a clean cloth soaked in water while the cats remained in the cage. 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 each contact with a cat.

2.3. Animals

This study was part of a research project designed to examine the effects of behavioral interventions on mucosal immunity and the respiratory health of cats rated as Anxious, Frustrated or Content upon admission. Between May and November 2010, cats that had been surrendered by their owner or brought in as strays by a humane officer, that were over 6 months old and free of clinical signs of upper respiratory disease (URD) such as mucopurulent nasal and/or ocular discharge, conjunctivitis (uni or bi-lateral), sneezing and / or coughing and injury, formed the pool from which cats (n =250) were obtained for this study. Of the 250 cats, 96 were assessed as Content upon admission and enrolled in the positive human interaction study (Table 1). Of these, 22 cats were taken out of the observation ward before the end of the 10-day observation period. In the Control group 7 cats were redeemed to owners (4,1,1,1 cats on days 2, 4, 5 and 7 respectively) and 7 cats were sent to isolation (3,1,1,2 cats on days 3, 4, 6, 7, 8 respectively). In the Treatment group 6 were redeemed to owners (1, 3, 2 on days 2, 3 and 5 respectively and 2 cats were sent to isolation on days 8 and 9, respectively.

Nine cats were sent to isolation for medical reasons and 13 were redeemed by their owner. Of the 74 cats that remained in the study for 10 days or more, 56 were adopted (average days to adoption = 31), 2 were euthanized (average days to euthanasia = 37) and 16 went to isolation (average days to isolation = 17). The 74 cats that remained in the experimental ward for 10 days were transferred by staff to an adoption area afterwards. The health and fate of all study cats still in the shelter was monitored for up to 40 days.

2.3.1. Physical examination, viral and bacterial cultures

Upon admission, cats were examined by a certified animal health technician (AHT) to determine the presence of clinical signs of upper respiratory disease such as mucopurulent nasal and/or ocular discharge; conjunctivitis (uni or bi-lateral) sneezing and / or coughing and injuries. They were vaccinated (Fel-O-Guard+3 Boehringer Ingelheim Ltd., Burlington, Ontario, Canada) and dewormed (Strongid® T. Pfizer, Quebec, Canada).

Cats were also examined daily by an AHT. Those with clinical signs of URD were sent to a medical isolation ward for treatment. Ocular and pharyngeal swabs were taken immediately following intake examination (Day 0) by the AHT. Subsequent swabs were obtained on days four and ten for all study cats still at the shelter, which did not apparently adversely affect their mood (which we defined as a persistent emotional state over 24 h). Saliva samples were analysed by real-time PCR assays (PCR oligonucleotides and protocols, IDEXX, Westbrook, Maine, USA, Burns et al., 2011). 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 for *Bordetella bronchiseptica*, *Chlamydophila felis*, *feline calicivirus*, *feline herpesvirus type 1* (FHV-1), H1N1 influenza virus and *Mycoplasma felis*. 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,

Table 1
Differences between Control and Treated cats arising from age, neutered status, gender and surrender type in a study on human-cat interaction in an animal shelter in Canada

	Control		Treated		Student's T test	*p*-value
Cat Characteristics	N	%	N	%		
Total	49	51	47	49		
Adult	30	61.2	26	55.3		
Juvenile	12	24.5	11	23.4		0.94
Senior	7	14.3	10	21.3		
Intact	20	40.8	21	44.7		0.86
Neutered	29	59.2	26	55.3		
Male	24	49.0	24	51.1		0.29
Female	25	51.0	23	48.9		
Owner-surrendered	14	28.6	16	34.0		0.96
Stray	35	71.4	31	66.0		

and a 384-well plate configuration. Samples were tested by quantitative real-time polymerase chain reaction (r-PCR). Prevalence of the bacteria and viruses determined in this study has been reported previously (Gourkow et al., 2013). Cats were considered to be shedding if they were PCR positive for one or more of the pathogens tested.

2.4. Behavioural observation on admission (day 0)

Following the admission examination, each cat was placed in a small wire cage covered with a towel and transported by staff to the experimental ward on the second floor of the shelter. The journey of 2 min did not require passing through any other cat housing units or dog areas. Cats were allocated to cages as available. Upon entering the room, 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.

A 1 h real-time video observation (from an adjacent room) commenced as soon as a cat was placed in a cage. This was followed by the experimenter entering the room and conducting a Human Approach Test, adapted from Kessler and Turner (Kessler and Turner, 1999) as follows: Step 1: the experimenter stood in front of the cage without interaction, no 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, 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). However, if cats responded aggressively (growling, hissing, attempts to scratch or bite), the door was closed immediately.

Following the observation period and the Human Approach Test, cats were assigned an emotional rating of Content, Anxious or Frustrated based on their overall responses (Tables 2 and 3). Of the 250 cats assessed upon admission (day 0), 96 were rated as Content, 139 cats were rated as Anxious and 15 were rated as Frustrated. Specifically, cats were rated as Content if they met the criteria for Contentment listed in Table 2 during the 1 h observation period (Day 0), and showed a positive response during the approach test (Table 3). These behavioural indicators had been previously validated with physiological correlates, S-IgA and cortisol, in 34 cats during their first week at an animal shelter (Gourkow et al., 2014a). In brief, 37 behaviors used in other studies for the assessment of welfare in shelter and household cats were initially selected as candidates for an index of emotions. Following observations, some behaviour with seemingly similar motivation and significant Spearman rank correlations were amalgamated and infrequently observed behaviours were removed. The 24 remaining behavior variables had been subjected to a principal component analysis producing a three dimensional model which was interpreted according to biplot methodology (Gabriel, 1971). The resulting multidimensional model represented two contrasting emotions, anxiety and contentment, indicative of high and low arousal of the emotional defence system respectively. A third dimension represented an emotion elicited by low arousal of the reward system consistent with frustration.

This paper reports the results of behavioural treatment of the cats rated as Content upon admission (day 0). The effect of gentling on Anxious cats (Gourkow et al., 2014b) and cognitive enrichment on Frustrated cats (Gourkow et al., submitted) are reported separately. The Content cats were alternately allocated to either a Treatment (n = 47) or Control group (n = 49) immediately after the emotional rating (day 0), in order of admission to the study.

2.5. Daily rating of moods

To examine changes in cats' moods over days, their behaviour was assessed using focal sampling from the videorecord (10 min per hour for 10 days). The results amalgamated over 24 h gave a total of 427 days of observation for the treatment group and 413 observation days for the Control group, allowing for the fact that cats that became sick where sent to the isolation ward were behaviour was no longer recorded. Moods were rated using the same emotion indicators as for the initial behaviour assessment. As described in Table 1, cats were rated as Content when spending $\geq$80% of awake time engaging in Contentment behaviours and <10% engaged in Frustration behaviour. Cats were rated as Anxious when they engaged in Anxiety behaviours $\geq$80% of awake time and <10% engaged in Frustration behaviours, <20% of awake time engaged in Contentment behaviours and were never observed lying on side. Cats were rated as Frustrated when spending $\geq$10% of awake time engaged in Frustration behaviours even when spending the rest of awake time engaged in Contentment behaviours.

2.6. Human interaction

Treatment was provided four times/d for 10 min sessions (commencing at 0600, 1100, 1600 and 2000 h) by the same experimenter (NG). 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 cage 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

Table 2
Criteria used for the rating of mood during the intake assessment (one hour observation) and for daily assessment of mood (10 min / hour) (adapted from Gourkow et al., 2014) in a study on human-cat interaction in an animal shelter in Canada

Mood / behaviour	Description
Content	**Behaviour observed >80% of awake time**
Sleep / rest	Lying down, relaxed body posture with eyes closed or semi closed.
Lie on side	Lying on flank, body and tail stretched, neck and ventral area exposed.
Front sit	Sitting upright at the front of the cage, calmly observing activities.
Groom	Licks body or paws, rubs head with paws (without chewing or pulling coat).
Eat / Drink	Takes food or water into mouth. Does not spill food or water around the cage.
Walk	High body posture, normal gait, functional locomotion to access areas of the cage (not on-going or repetitive).
Friendly	Approaches and interacts with humans in an amicable manner.
Rub	Rubs body or head on objects and cage door.
Absent	*All Anxiety and Frustration behaviours.*
Anxious	**Behaviour observed >80% of awake time**
Flat	Low body posture when lying down, sitting or standing for locomotion.
Freeze	Remains completely immobile, body and head flattened, eyes wide open.
Hide	Body fully or partially hidden under bedding, behind or in litter box.
Crawl	Slow locomotion while keeping body flattened close to ground.
Startle	Sudden & brief tensing of the body.
Retreat	Avoidance of human usually by retreating to the back of cage (often accompanied by lip licking*).
Inhibition	*Sleep, grooming, locomotion, drink / eat (particularly in the presence of humans.*
Absent	*All other Contentment and all Frustration behaviours.*
Frustrated	**Behaviour observed >10 % of awake time**
Meow	Persistent and loud meowing, not related to anticipation of food
Escape bouts	Engaging in one or more behaviour in a repetitive or persistent pattern: standing on hind limbs, pawing wall or floor, pushing paw through door, pushing on door latch, hanging on cage door with body inverted, biting or licking cage bars*.
Scan	Persistent visual scanning of all areas of the cage
Push	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. Pressing body or head on cage door.
Pace	Persistent, repetitive and rapid locomotion at cage door or circle pattern.
Aggression	Unpredictable, short burst of aggression such as biting or scratching during otherwise friendly interaction with a human *(not accompanied by defensive behaviour or vocalizations*).
Absent	*All Anxiety behaviours and lie on side.*

*Lip licking and bar biting were not included in the original study (Gourkow et al., 2014) but were added to this study as they were exclusively and frequently observed together with other anxiety and frustration behaviours, respectively.

Table 3
Criteria used for rating of responses to humans (Positive Human Interaction and the Human Approach Test) as positive or negative in a study on human-cat interaction in an animal shelter in Canada

Response type	Response sub-type	Description of responses
Negative	Defensive aggression	Hissing, growling and paw strike
	Defensive retreat	Flattens body and ears, freezes or retreats
Positive	Calm	Relaxes body, lowers the head when petted between ears, raises the head when petted on the chin. Absence of defensive aggression or retreat
	Friendly	Stands close to, or approaches the experimenter; alternatively remains in a sitting or lying down posture, rubs themselves on the experimenter's hands, maintains themselves in a relaxed body posture; may also walk to the food bowl and eat during treatment

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. The responses of cats to Treatment or Control were rated as positive or negative, according to behavioural indicators outlined in Table 3.

2.7. Faeces collection and S-IgA assays

Stools were collected whenever produced, and were weighed and immediately frozen at -40 °C. Samples were analysed for IgA concentrations, using the method described in Gourkow et al. (2014a). In brief, samples were extracted and vortexed until homogenised. Following centrifugation, addition of a protease inhibitor and placement in ELISA plates, IgA values were obtained in a multilabel plate reader. Coefficients of variability were 5.4% and 9.1% for intra and inter assays, respectively, within the accepted limits of 10 and 15%, respectively (Anon, 2014)

2.8. Statistical analyses

Results were considered significant at alpha $\leq$0.05. Student's t tests were used to determine if there were significant differences in characteristics (age, gender, sterilization status, and source) of Control and Treated cats at time of enrolment. All continuous variables compared were tested for equal variance with the Bartlett test and residuals tested for normal distribution by the Wilk-Shapiro test.

2.8.1. The effect of treatment on daily mood.

A Poisson regression analysis was used to compare daily mood ratings for Treated and Control cats that had been rated as Content on arrival (Day 0). 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, Frustrated) and number of cats rated as Content each day. 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 overdispersed and did not have an excessive number of zeros.

2.8.2. Cat characteristics and daily mood

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).

2.8.3. Influence of mood and human interaction 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 $\log_e$ transformed to achieve a normal distribution. A General Estimating Equation (GEE) was used to determine if there was a significant difference in S-IgA levels depending upon time (days) 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 the 24 hours rating period) were removed from the analysis.

2.8.4. The effect of human interaction on incidence of viral and bacterial shedding

Fisher's exact test was used to determine if the rate of shedding on admission was significantly different between Treated and Control groups. GEE was used to determine if the rate of shedding changed over time.

2.8.5. The effect of human interaction on incidence of URD

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 ratios, CI and corresponding P-values are reported). A Cox-Proportional Hazards model was used to compare the incidence of URD in Treated and Control groups. Additionally, GEE were used to determine 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. Results

3.1. Baseline cat characteristics

Cat characteristics did not differ significantly between the Treated and Control groups: age (seniors, adults, and juveniles, $p = 0.94$), gender (male, female $p = 0.29$), sterilization status (intact, neutered, $p = 0.86$) and source (owner-surrendered, strays, $p = 0.96$) (Table 1). All cats responded positively to the Human Approach Test, therefore no statistical analysis was performed.

3.2. Effect of human interaction on daily mood

Between days 1 and 10, Treated cats were more likely to sustain their contented mood than Control cats (Poisson Values IRR =1.13, CI: 0.98-1.30, $P < 0.0001$; Fig. 1). Specifically, 384 out of 427 days of observation (90.0%) were rated as Content for Treated cats versus 345 days of 413 (84%) for Controls. Control cats were also more likely to experience the onset of negative emotions (Frustration or Anxiety) than Treated cats (IRR: 0.61, CI: 0.42-0.88, $P = 0.007$; Fig. 2).

3.3. Cat characteristics and daily mood

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

3.4. Influence of mood and treatment on S-IgA levels

There was a significant difference in the mean number of stools analysed for the two groups (Control 3.9 ± 1.9/cat, Treated 4.7 ± 1.7/cat; $P = 0.03$). There was a trend for a reduction in S-IgA concentration ($\log_e$ μg/g) over time in both groups (Control $P = 0.05$, Treated $P = 0.06$, Fig. 3). Treated cats had higher S-IgA levels than Control cats (7.28 ± 0.7 Vs 6.74 ± 0.5 $\log_e$ μg/g, or 1451 Vs 846 μg/g, $P < 0.0001$). S-IgA was higher for Treated cats that were rated as Content ($7.52 \pm$n0.5 $\log_e$ μg/g or 1846 μg/g) than for Treated cats that became Frustrated or Anxious (7.24 ± 0.3 $\log_e$ μg/g or 1394 μg/g, $P < 0.001$). or Control cats that were rated as Content (7.01 ± 0.7 $\log_e$ μg/g or 1108 μg/g, $P < 0.001$). Response to treatment (positive/negative) did not affect S-IgA levels ($P = 0.18$). Overall, there was no significant difference in S-IgA attributable to source, gender or sterilization status ($P \geq 0.14$). However, there was a trend for S-IgA concentration to increase with age (Adult 6.65 ± 0.6 $\log_e$ μg/g or 772 μg/g, Juvenile 6.70 ± 0.7 $\log_e$ μg/g or 812 μg/g, and Senior 6.77 ± 0.7 $\log_e$ μg/g or 871 μg/g; $P = 0.07$).

3.5. Effect of human interaction on incidence of viral and bacterial shedding

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 $P = 0.006$). However, there was an increase in shedding over time in Controls cats (22%, 36% and 61% on days 1, 4 nad 10, respectively), but not Treated cats (35%, 26% 32% on days 1, 4 and 10, respectively) (GEE $P = 0.006$).

3.6. Effect of human interaction on incidence of URD

Control cats were more likely to develop URD over time compared to Treated cats (Fig. 4, HR: 2.94, CI: 1.30-6.67, $P = 0.01$). Response to treatment was associated with URD status. In no URD group there were 317 (96%) positive responses and 14 (4%) negative responses. In the URD group there were only 72 (83%) positive responses and 15 (17%) negative responses ($P = 0.008$). Of the 49 cats in the control group, 17 cats (34.7%) developed URD. Of the 47 cats in the treatment group, 9 cats (19.1 %) developed URD. Thus there was a reduction of 15.6 % in URD in cats receiving treatment.

4. Discussion

In humans, sustained contentment (or happiness) is associated with enhanced immune protection (Barak, 2006). In this study, we tested the hypothesis that interactions with a familiar human could sustain a contented mood in cats rated as Content upon admission to an animal shelter. We also hypothesized that sustained contentment in cats might stimulate secretion of IgA and reduce incidence of URD throughout their stay at the shelter. Our hypothesis was based on findings from our previous study which showed that Content cats had significantly higher S-IgA values than Anxious and Frustrated cats (Gourkow et al., 2014a). In both the previous study and this one, contentment was indicated by behaviours such as sitting at the front of the cage, lying on their 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

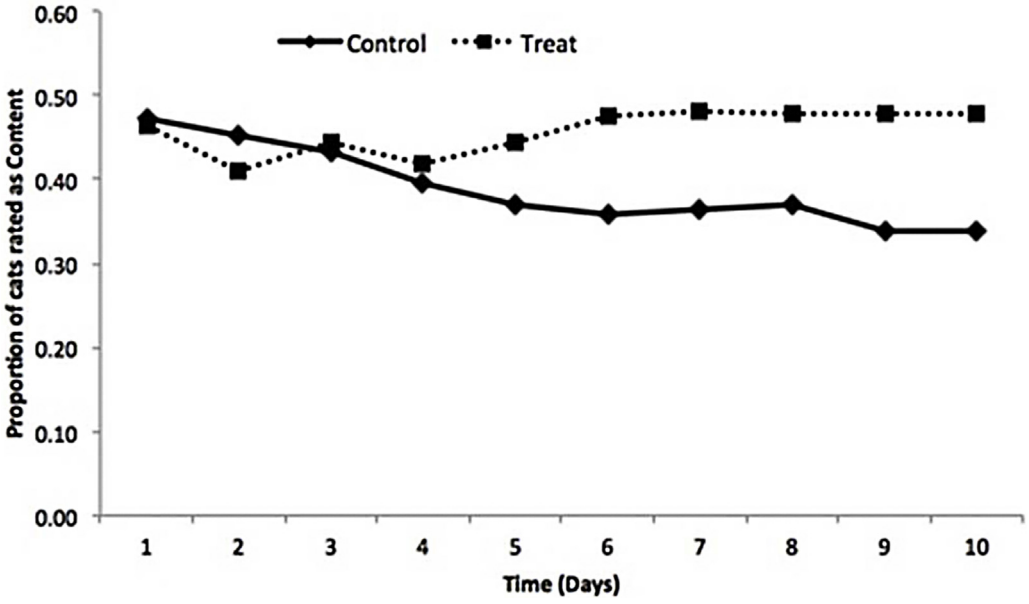

Fig. 1. Proportion of cats rated as Content from day 1 (Treated n=47, Controls n=49 cats) to day 10 (39 and 35 cats, respectively) in a study on human-cat interactions in an animal shelter in Canada (SEM 0.07).

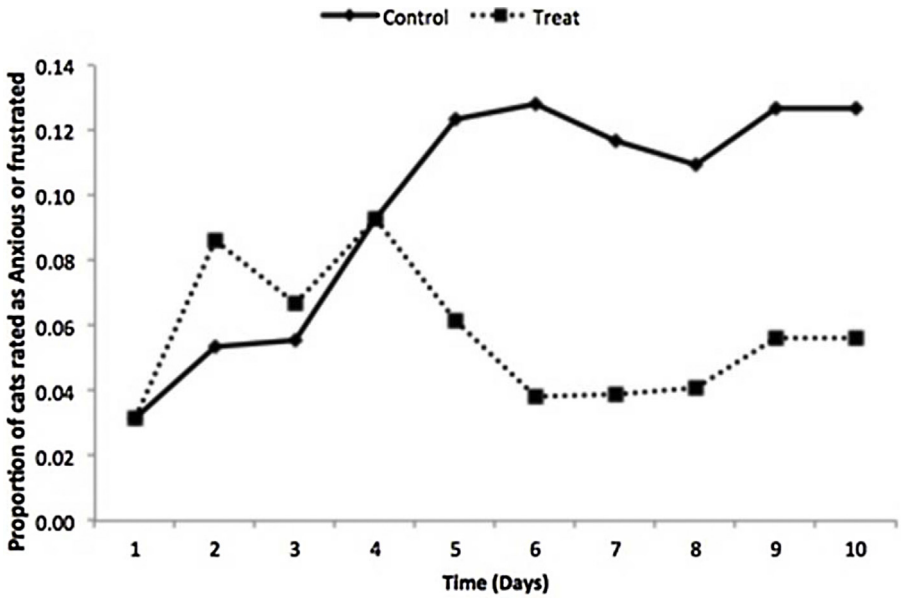

Fig. 2. Proportion of cats rated as Anxious or Frustrated groups from day 1 Control (n=49) and Treated n=47) to day 10 (35, and 39 cats, respectively) in a study on human-cat interaction at an animal shelter in Canada.

research project, 38% were rated as Content upon admission, compared to 56% and 6% rated as Anxious and Frustrated, respectively; supporting the notion that entering an animal shelter induces stress in cats (McCune, 1992; Rochlitz et al., 1998a; Rodan, 2010).

Our findings indicate that interactions with a familiar human facilitate sustained contentment, whereas depriving cats of such interactions results in onset of frustration or anxiety. Similar results have been reported for Content cats deprived of environmental enrichment (hiding boxes) (Kry and Casey, 2007). The authors found that cats showing calm and content behaviours during the first week at the shelter were attempting to hide behind the litter tray by week two. These studies suggest that to sustain positive affect in shelter cats some form of enrichment must be provided.

Our findings showed that Treated and Control cats experienced a decrease in S-IgA over time, suggesting that all may have experienced some stress. However, S-IgA remained higher in Treated cats throughout the study. The positive effect of interactions with a familiar human was most evident from the higher S-IgA level in cats with sustained contentment in the Treated group compared with the Control group and that the activities provided, such as petting, brushing or playing, have an added benefit for immunity. Similarly, in humans, pleasant activities such as back rubs (Groër et al., 1995), relaxation training (Taniguchi et al., 2007) and positive vocalizations (Watanuki and Kim, 2005) stimulate S-IgA. It must be acknowledged that lower S-IgA in the Control group may have been due to the presence of a human close to the cage, even though they were withholding eye or vocal contact. In dogs, removal of attention

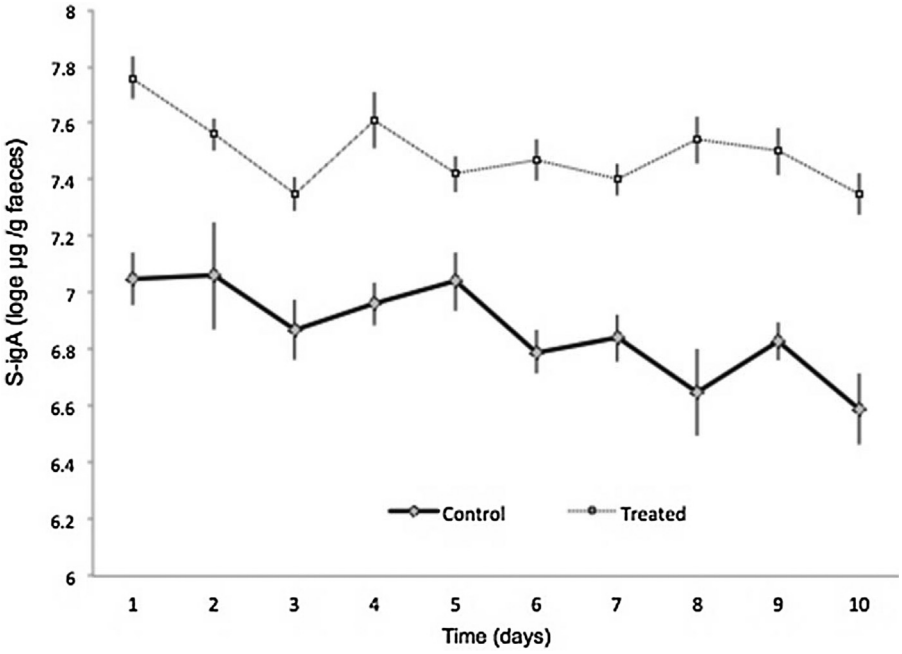

Fig. 3. Secretory immunoglobulin A (?SE) over days for treated ($n = 47$) and control cats ($n = 49$) in a study on human-cat interaction at an animal shelter in Canada.

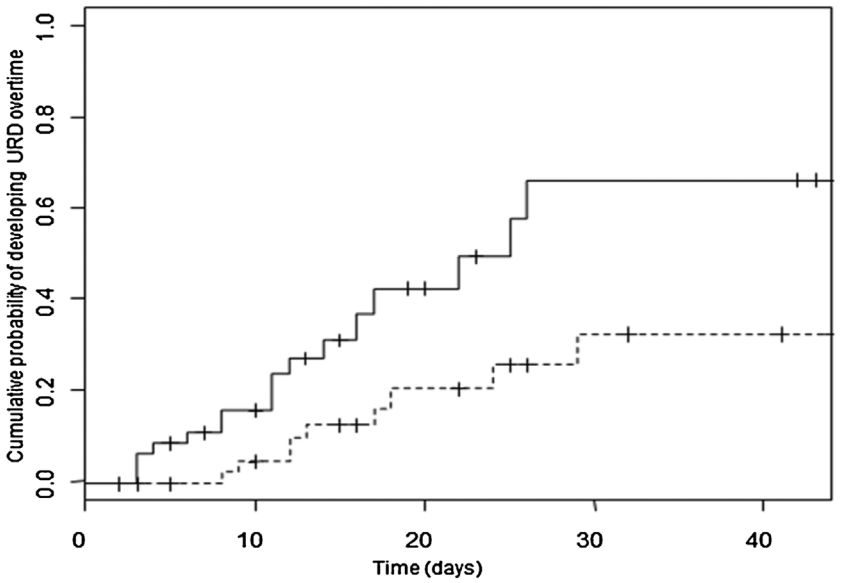

Fig. 4. Cumulative probability of treated and control cats developing URD over time (40 days) in Treatment (- - - n=47) and Control (— n=49) Groups, derived from Kaplan-Meier Failure Curves, in a study on human–cat interaction at an animal shelter in Canada.

is commonly used as a means to extinguish an undesired behavior (Browne et al., 2011). Similarly, the withholding of vocal interactions and petting causes an increase in adrenal output in laboratory cats (Carlstead et al., 1993).

Our findings indicate that intact cats were more likely to sustain a positive mood (contentment) than neutered cats. Studies have shown equivocal results regarding stress levels of stray cats (more of which are intact) in animal shelters. Dybdall et al., (2007) reported that strays coped better, whereas McCobb et al., (2005) found no difference in adrenal output between strays and owner-surrendered cats (most of which are neutered). It is possible that intact cats (even when owned) are given access to an outside area more than neutered cats, allowing them to be exposed to a wider range of stimuli. It has been reported that well-socialized cats show faster adaptation to the shelter environment (Kessler and Turner, 1999). On the other hand, intact cats tend to roam over a wider territory and as a result may experience more frustration in response to confinement.

Our hypothesis that sustained contentment contributes to respiratory health in cats was well supported by the current findings. In addition to an increase in viral shedding among the control cats, we found 10% more shedders by day 4 and 29% more by day 10 among Control cats. According to Pedersen and colleagues (2004), the onset of shedding shortly after entering indicates 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 whereas later onset

of non-carriers was considered infections contracted at the shelter. It is well documented that stress is a major factor in reactivation of subclinical infections, particularly feline herpesvirus (Gaskell et al., 2007; Gaskell and Povey, 1979). Thus we may interpret the current data to mean that human interaction treatment 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 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 (Hurley, 2011), 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.

Limitations of the Study

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 (Kostarczyk and Fonberg, 1982). However, it has also been proposed that shelter cats may lose interest in interactions due to habituation to the presence of a human (Hoskins, 1995). Thus, interactions with different people may be beneficial for some cats to prevent boredom.

5. Conclusions

Human interaction treatments 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.

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