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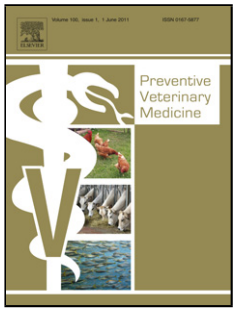

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Effect of gentle stroking and vocalization on behaviour, mucosal immunity and upper respiratory disease in anxious shelter cats

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ABSTRACT

Emotional, behavioral, and health benefits of gentle stroking and vocalizations, otherwise known as gentling, 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. The cats' mood, or persistent emotional state, was rated daily for 10 d as Anxious, Frustrated or Content. Gentled cats were less likely to have negatively valenced moods (Anxious or Frustrated) than Control cats (Incidence Rate Ratio [IRR] =0.61 CI 0.42-0.88, *P* =0.007). Total secretory immunoglobulin A (S-IgA) was quantified from faeces by enzyme-linked immunosorbent assay. Gentled cats had increased S-IgA (6.9 ±0.7 $\log_e$ µg/g) compared to Control cats (5.9 ±0.5 $\log_e$ 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, $\log_e$ µg/g), compared with those that responded negatively (6.14 ±0.8, $\log_e$ µg/g). Combined conjunctival and oropharyngeal swab specimens were tested by quantitative real-time polymerase chain reaction (rPCR) 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 Control cats (23%, 35%, 52% on days 1, 4 and 10, respectively), but not in gentled cats (32%, 26%, 30% on days 1, 4 and 10, respectively) (*P* =0.001). Onset of upper respiratory disease was determined by veterinary staff based on clinical signs, in particular ocular and/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. Introduction

In humans, the relationship between negative life events and susceptibility to diseases, such as the common cold, is well established (Cohen et al., 1991; Evans and Edgerton, 1991; Pressman et al., 2005). In cats too, a stressful event, such as entering an animal shelter, can reactivate subclinical conditions (e.g. feline herpesvirus type 1) (Gaskell et al., 2007) and inhibit the production of mucosal antibodies, particularly secretory immunoglobulin A (S-IgA) (Gourkow et al., 2014), resulting in increased susceptibility to pathogens that cause Upper Respiratory Disease (URD) (Hannant 2002). Hence, the management of emotional stress may be of clinical importance in managing respiratory disease (Griffin, 2012; Hurley, 2006; McMillan, 2002; McMillan, 2005).

Physical contact between cats, such as allogrooming and allorubbing, facilitates social bonding (Crowell-Davis et al., 2004; van den Bos, 1998); and petting seems to serve a similar purpose in the cat/human relationship (Bernstein, 2007). 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 (Mertens, 1991). 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 (De Luca et al., 1992). Petting can reduce the heart rate in dogs (Kostarczyk and Fonberg, 1982) and horses (McBride et al., 2004); and reduce fear of humans in cows (Breuer et al., 2003), rabbits (Csatádi et al., 2005) and dogs (Coppola et al., 2005; Hennessy et al., 1998; Luescher and Tyson, 2009; Normando et al., 2009). Petting and therapeutic massage of cats are believed to reduce stress associated with chronic pain (Robertson et al., 2010), and five min of petting can reduce arterial blood pressure (Slingerland et al., 2008). 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 (Carlstead et al., 1993).

Despite the documented benefits, in some cats even gentle petting may induce aggression (Rodan, 2010). This is marked by tail twitching, increased muscle tension, leaning away, flattened ears, horizontal retraction of the lips, and hissing (Hunthausen, 2006). 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 (Rodan, 2010). In addition, approximately 20% of cats are thought to be genetically predisposed towards defensive behavior to humans, which is not affected by prolonged socialization (Adamec et al., 1983; McCune, 1995; Reisner et al., 1994). 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. However, petting in the temporal region (between the eyes and ears) rather than in the caudal region (Soennichsen and Chamove, 2002), and delivery using short strokes with circular movements (Tellington-Jones, 2003), 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 (Caroprese et al., 2010). S-IgA is the most abundant mucosal antibody and is necessary for protection against pathogens that can be inhaled or ingested (Stokes and Waly, 2006). 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 (Edinboro et al., 1999; Foss and Murtaugh, 2000). Emerging attitudes in

veterinary medicine emphasize the importance of addressing negative emotional states in animals, as they may compromise health (Griffin, 2012; McMillan, 2005). The hypothesis examined in this study was that suitable gentling of cats in a shelter would reduce anxiety and increase S-IgA, with a concomitant reduction in URD. Epidemiological aspects of this study have been reported separately (Gourkow et al., 2013).

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 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 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 30g of wet food (Science Diet, Hill's Pet Nutrition, Inc. ®/™ Topeka, KS, U.S.A.). Fresh water was provided ad libitum. Feeding was undertaken by the experimenter, shelter staff or volunteers.

The cat housing in the experimental ward consisted of 20 stainless steel cages (76 x 76 x 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 CCD25M crystal-View Super Hi-Res ICR IR Camera SLED w/9-22mm 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. 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.

154 *2.3 Animals*

155 This study was part of a research project designed to examine the effects of behavioral
156 interventions on mucosal immunity and the respiratory health of cats rated as Anxious,
157 Frustrated or Content upon admission. Between May and November 2010, cats that had been
158 surrendered by their owner or brought in as strays by a humane officer, that were over 6 months
159 old and free of clinical signs of upper respiratory disease (URD) (Table 1) and injury formed the
160 pool from which cats (n =250) were obtained for this study. Of the 250 cats, 139 were assessed
161 as Anxious upon admission and enrolled in the Gentling study. Of these, 37 cats were removed
162 from the study before day 10 (9, 7, 3, 2, 5, 6, 2, 3 cats left the study on days 2, 3, 4, 5, 6, 7, 8
163 respectively). Three were euthanized, 25 were sent to isolation for medical reasons and 9 were
164 redeemed by their owner. Of these, 102 cats remained in the study 10 days or more, 64 were
165 adopted (average days to adoption = 34), 10 were euthanized (average days to euthanasia = 34)
166 and 28 went to isolation (average days to isolation = 20). Of the cats euthanized, all were for
167 medical reasons, including 1 for untreatable URD. The 102 cats that remained in the
168 experimental ward for 10 days were transferred by staff to an adoption area afterwards. Data on
169 cats' behavior was collected for the first 10 days at the shelter. Cats' health and fate was
170 recorded for these ten days and then continued to be monitored for up to 40 days.

171

172 *2.3.1 Physical examination, viral and bacterial cultures*

173 Upon admission, cats were examined by a certified animal health technician (AHT) to determine
174 the presence of clinical signs of upper respiratory disease (Table 1) 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 (Table 1) were removed from the study and 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 (defined as persistent emotional state over 24 h)(Fig. 1). 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, and a 384-well plate configuration. Samples were tested by quantitative real-time polymerase chain reaction (r-PCR).

2.4 Behavioral observation on admission (day 0)

Following examination, each of the 250 cats were 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, which produced an approximately random distribution to

197 the 20 cages with cameras. Upon entering the room, staff lifted each cat into their cage (covered
198 with a towel prior to lifting if they were growling or hissing) and immediately exited the room.
199 A 1 h real-time video observation (from an adjacent room) commenced as soon as a cat
200 was placed in a cage. This was followed by the experimenter entering the room and conducting a
201 Human-Approach Test, adapted from Kessler and Turner (Kessler and Turner, 1999) as follows:
202 Step 1: the experimenter stood in front of the cage without interaction, no eye contact or verbal
203 greeting (2 min); Step 2: the experimenter talked to the cat using a high-pitched gentle tone, and
204 had some eye contact, with eyes half closed (1 min); Step 3: the same procedure was repeated
205 with the door open, followed by an approach of the hand so that it was near the cat (2 min).
206 However, if cats responded aggressively (growling, hissing, attempts to scratch or bite), the door
207 was closed immediately.
208 Following the observation period and the approach test, cats were assigned an emotional
209 rating of Anxious, Frustrated or Content based on their overall response in these (Table 2). Of
210 the 250 cats assessed upon admission (day 0), 139 cats were rated as Anxious, 15 were rated as
211 Frustrated and 96 were rated as Contented. Specifically, cats were rated as Anxious if they met
212 the criteria for Anxiety listed in Table 2 during the 1h observation period, i.e. if they attempted to
213 hide under bedding or behind the litter box while keeping a flattened posture and if they further
214 retreated, flattened their body or became aggressive (hissing, growling, attempts to scratch or
215 bite) during the approach test. These behavioral indicators had been previously validated with
216 physiological correlates, S-IgA and cortisol, in 34 cats during their first week at an animal shelter
217 (Gourkow et al., 2014). In brief, 37 behaviors used in other studies for the assessment of welfare
218 in shelter and household cats were initially selected as candidates for an index of emotions.
219 Following observations, some behaviors with seemingly similar motivation and significant

Spearman rank correlations had been amalgamated and infrequently observed had been 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 Anxious cats, with treatment of the other two groups reported separately (Gourkow et al., in preparation). The Anxious cats were alternately allocated to either a Gentling (n = 70) or Control group (n = 69) immediately after the emotional rating (day 0), in order of admission to the study. Although there were more adult cats in the Control than Gentled group, there were no significant differences in sex (male, female, P = 0.10), source (owner-surrendered, strays P = 0.19) or sterilization status (neutered, intact, P = 0.09) between cats in the Gentled and Control groups (Table 3).

2.5 Daily rating of moods

To examine changes in moods (emotional state over a 24 h period) over days, focal sampling of behavior was done using the videorecord (10 min per hour for 10 days). Mood scores per 24 hours were assigned based on target behaviours being observed ≥ 80% of the time for Anxiety and Contentment and ≥10% of Frustration. The results amalgamated over 24 h gave a total of 613 cat days for the Gentling group and 565 cat days for the Control group, allowing for the fact that cats that became sick were removed from the study. Moods were rated using the same emotion indicators as for the initial behaviour assessment (Table 2).

243

244 *2.6 Gentling*

245 Gentling is defined for the purposes of this study as gentle stroking of the head and neck area of
246 the cat together with gentle vocalization. It was provided to each cat in the same order each day
247 for 10 min, 4 times per d (0600, 1100, 1600 and 2000 h), by the same experimenter (NG) each
248 day (with the exception of a few days when a trained research assistant and a volunteer
249 performed the Gentling). The exact time that each cat was treated varied slightly depending on
250 the number of cats to be treated each day. All cats to be gentled were first verbally greeted using
251 a high-pitched gentle tone for 30s, with the door closed. The door was then opened with an
252 approach of the experimenter's hand offered for the cat to sniff. Gentling methods were modified
253 to differentially accommodate cats' initial responses:

254

255 *2.6.1 Gentling: Anxious cats*

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

263

264 *2.6.2 Modified Gentling 1: Aggressive response*

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 round 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 for anxious cats 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 response

If the cat responded positively (stood, walked, rubbed on experimenter, or walked to the food bowl and ate), gentling was not interrupted and not limited to the head area.

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 either positive or negative, according to behavioral indicators recorded and viewed on camera immediately after each treatment (Table 2).

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. (2014). 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 ≤ 0.05. Fisher's exact test was used to determine if there were significant differences in cat characteristics of Control and Gentled cats at time of enrolment.

2.8.1 Behavior upon admission

Chi-square test was used to determine if there was a significant difference in behaviour upon admission. Behaviour was classified as either defensive retreat or aggression (hissing, growling, attempt to bite or scratch).

2.8.2 The effect of treatment on daily mood

A Poisson regression analysis was used to compare changes in daily mood rating for Gentled and Control cats that had been rated as Anxious on arrival. For all Poisson regression analyses, 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.

2.8.3 Cat characteristics and daily mood

Generalized estimating equations (GEE) were used to determine if the cat characteristics were significant predictors of daily mood , these being appropriate if there are correlations between observations (in this case days for each of the cats). The test used the binomial positive responder/ negative responder to treatment at each time point as the response variable, with age, source, sex and sterilization status as explanatory variables.

2.8.4 Positive and Negative Responses to Gentling

Generalized estimating equations (GEE) were used to determine if the daily mood was a significant predictors of response to gentling treatment, The test used the binomial positive responder/ negative responder to treatment at each time point as the response variable, with daily mood as the explanatory variable.

2.8.5 The influence of mood and Gentling treatment on S-IgA levels

A t-test was used to determine if there was a mean difference in the number of stools between treated and control cats. Variables were tested for equal variance with the Bartlett test and residuals tested for normal distribution by the Wilk-Shapiro test. S-IgA values were $\log_e$ transformed to achieve a normal distribution, and a GEE was used to determine if there was a significant difference in S-IgA levels (response variable) over days (explanatory variable).

Additionally, GEE were used to determine if S-IgA levels differed in cats that were positive versus negative responders to treatment (Gentling), and if responses to gentling varied according to age, source, sex, and sterilization status.

Mood ratings on days for which there were no available stools (within 24 hours of rating) were removed from the analysis of S-IGA.

2.8.6 The effect of gentling on incidence of viral and bacterial shedding

Fisher's exact test was used to determine if gentling affected whether a cat was recorded as shedding on each of the days affected. The same test was used to examine whether gentling affected the development of clinical signs of URD over the ten days (Table 1). A GEE was used to determine if shedding status (yes/no) changed over time.

2.8.7 The effect of gentling and cat characteristics on incidence of URD

A Cox-Proportional Hazards model was used to compare the incidence of URD between Gentled and Control groups over time (Hazards ratio, CI and corresponding p-value are reported). 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). Additionally, the effects of the factors age, sterilization status and sex on URD outcome (yes/no) was analyzed by binary logistic regression with a logistic model.

3 Results

3.1 Behavior upon admission

Of the cats rated as Anxious (n =139) upon admission (day 0), 81.3% (n =113) responded with defensive retreat and 18.7% (n =26) responded with aggression (hissing, growling, attempt to bite or scratch) (Chi-square value 54.4, P < 0.001).

3.2 The effect of treatment on daily mood

Between days 1 and 10, Control cats were more likely than Gentled cats to be rated as Anxious or Frustrated (Poisson Values IRR =0.61, CI 0.42-0.88, *P* =0.007; Fig1). 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.

3.3 Cat characteristics and daily mood

Age (P = 0.18), sex (P = 0.53) and sterilization status (P = 0.68) were not significant predictors of daily mood scores. However owner-surrendered cats were 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).

3.4 Positive and Negative Responses to Gentling

Within the Gentled group, the likelihood of negative compared with positive responses was dependent on the cats' mood that day: cats were 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% positive response respectively) (Table 5, P

379 <0.0001). Sex (P = 0.72), sterilization status (P = 0.25), age (P = 0.35) and source (P = 0.26)
380 were not significant predictors of response to treatment.
381
382 *3.5 The influence of mood and Gentling treatment on S-IgA levels*
383 Coefficients of variability of the IgA assay were acceptable, being substantially less than
384 the recommended 10 and 15% (Anon, 2014). There was no significant difference in the mean
385 number of stools analysed between the treatment groups (Control 3.8 ±1.6; Gentled 4.1 ±1.7, *P*
386 =0.24). S-IgA was higher in Gentled than Control cats (6.9 ± 0.7Vs 5.9 ± 0.5 $\log_e$ µg/g,
387 respectively), and a significant increase over days was found in both groups (GEE, *P* <0.0001)
388 (Fig.2). Between days 1 and 10, S-IgA values were greater for cats rated Content (7.0 ± 0.7 $\log_e$
389 µg/g) than those rated Anxious (6.6 ± 0.7 $\log_e$ µg/g) or Frustrated (5.9 ±0.4 $\log_e$ µg/g)(*P*
390 <0.0001). S-IgA values were also greater for Gentled Content cats than Content Control cats
391 (Gentled 7.0 ± 0.7; Control 6.3± 0.7 µg/g; *P* <0.001). Furthermore, S-IgA was significantly
392 greater for positive than for negative responders to gentling (7.0 ± 0.6 Vs 6.1 ± 0.8 $\log_e$ µg/g,
393 respectively, Table 6). There was no significant effect of source (P = 0.89), age (P = 0.10), sex
394 (P = 0.17) or sterilization status (P = 0.08) on S-IgA.
395
396 *3.6 The effect of gentling on incidence of viral and bacterial shedding*
397 *Mycoplasma felis* was the dominant organism detected (21% of cats), with some presence of
398 feline calicivirus, feline herpesvirus-1, and *Bordetella bronchiseptica* (approximately 2% of cats
399 each) (Gourkow et al., 2013b). The Gentling and Control groups did not differ in pathogen
400 shedding rate 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) (GEE, $P = 0.001$).

3.7 The effect of gentling and cat characteristics on incidence of URD

Control cats were 2.37 (HR; CI 1.35-4.15) times more likely to develop clinical URD over time than cats that received the Gentling treatment ($P < 0.0001$; Fig.3). The onset of clinical URD occurred significantly earlier for Control (mean 8.8 ±11.7 d) than Gentled cats (mean 18.5 ±5.6 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%) ($P < 0.0001$). There was no significant effect of age (P = 0.28 and 0.53 for juveniles and seniors Vs adults, respectively), sex (P = 0.29) or sterilisation status (P = 0.10). However, stray cats were more likely to get URD (26/48) than owner surrendered cats (27/91)(Odds ratio 5.0, CI 1.9-13.1, P = 0.001).

4. Discussion

Alleviating emotional pain is of clinical importance pursuant to the Veterinarian Oath (McMillan, 2002). Positive interactions with humans are a valued activity for cats in homes, whether they are allowed access to the outside or kept strictly indoors (Mertens, 1991). The current findings indicate that positive human interaction, in the form of gentling, can enhance emotional wellbeing and 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 and those responding positively had an even greater increase in S-IgA. Similar effects have been

observed in dogs, such as decreased heart rate and cortisol being more pronounced in dogs that display a friendly response towards the handler (Kostarczyk and Fonberg, 1982). Although these data confirmed previous findings (Gourkow et al., 2014) that Content cats produce more S-IgA than Anxious cats. In the current study, Content cats that were gentled showed a higher concentration of S-IgA compared to Content cats in the control groups. 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 (Groer et al., 1994). However, salivary IgA only increased in the group receiving the back rub. The specific effect of gentling on S-IgA, in addition to its positive effect on emotions, is of unknown aetiology. Gentling may induce changes in physiology that enhance mucosal immunity. In rats (Kurosawa et al., 1995) and dogs (Odendaal and Meintjes, 2003), gentle petting increases oxytocin, a neurochemical known to have benefits for wellbeing (Handlin et al., 2012; Plata-Salaman, 1989; Yang et al., 2010). In addition, it has been found in shelter cats that positive interactions with one person seem to increase positive responses to unfamiliar people (Hoskins, 1995). In rats, gentling reduces fear during subsequent exposure to a fear-provoking, open-field test (Hirsjärvi et al., 1990). Thus, gentling by one person may mitigate the effects of stressful encounters with various staff, such as during routine cleaning of the cage.

Cats too aggressive to handle were provided with mechanical gentling using an extendable stick equipped with a rubber tip. This technique produced a rapid decrease in Anxiety (and aggression), which in turn was associated with an increase in S-IgA production. In animals with a tendency to fear humans, such as sheep (Grandin, 1989; Grandin et al., 1986) and cattle (Grandin, 1992), mechanical restraint has a calming effect, compared to being handled by a

447 human. These findings have important implications for the welfare of fearful cats in institutional
448 settings. In North American shelters (and probably worldwide), staff are called upon to
449 determine if cats showing fear are likely to be feral, because they cannot be socialized past the
450 age of 3 months (Evans, 1999), or if they are socialized, but fearful, non-feral cats. This is a
451 difficult task, and cats classified as feral are routinely euthanized following a legal holding
452 period of (usually) 3 or 4 days (Slater et al., 2010). According to our observations, 18% of the
453 cats in this study would have been candidates for euthanasia within that holding period, based on
454 their aggressive response to the Human-Approach Test and to gentling. However, our research
455 protocol required all cats to be kept for 10 days prior to staff making an adoption/euthanasia
456 decision (with the exception of euthanasia for medical reasons), during which time aggressive
457 cats received mechanical gentling if they could not be safely handled. Among the gentled cats,
458 none responded with aggression after day 6. Thus, a 3 to 4 day holding period may not be
459 sufficient to differentiate non-feral from feral cats.

460 Our data suggests that emotional stress may induce viral reactivation in cats with
461 subclinical infections (Dawson et al., 2004). This is suspected because the clinical symptoms in
462 some cats as early as day 4 were severe, even though none had clinical signs on day 0.
463 Reactivation of a subclinical infection would be possible within this time frame, but a novel
464 infection would be unlikely. We observed an increase in shedding by day four in Control, but not
465 Gentled cats. It has been suggested (Pedersen et al., 2004) that the onset of shedding within a few
466 days at the shelter may be due the reactivation of a latent infection rather than infection
467 contracted on-site. Non-gentled cats also showed significantly higher incidence of URD, with
468 onset of clinical signs occurring sooner than for gentled cats that became sick. In both cases, our
469 conclusions are in accordance with researchers who propose that management of mental health

should be part of disease management practices in shelters (Dinnage et al., 2009; Griffin, 2012; Hurley, 2005). 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. It has been proposed that for humans (Diener and Emmons, 1985) and veterinary species (Griffin, 2012), interventions should address any specific emotional problem that may be affecting health. Gentling can reduce anxiety and the fear response observed in some cats when approached by reducing arousal of the emotional defence system; however, it likely does not address other moods such as frustration, for which underlying causes are behavioral restriction, non-reward or unpredictable appetitive events (Amsel, 1958; Latham and Mason, 2010; Lyons et al., 1997; Mills, 2009).

Limitation of the study

The anxiety emotional index developed in our previous study and used in this study appeared to accurately identify Anxiety in shelter cats. However, variation in emotional arousal (intensity of emotional response) cannot be determined by the indices in their current form. Therefore the increased S-IgA found in cats responding negatively to gentling may have been due to a decrease in emotional arousal that was not sufficient to classify cats as contented based on behavioural observations but sufficient to stimulate S-IgA. Further research to determine the effects of gentling on mucosal immunity according to various levels of arousal within each emotional classification may be of clinical importance.

Further, in this study, the gentling was consistently provided by the same experimenter (with the exception of a few days where cats were gentled in the same way by a trained research

assistant and a volunteer). The effect of familiarity was therefore not separated from the effect of gentling alone. Further research is needed to determine if the familiarity of the person providing gentling is important to the cats. 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. Two further limitations were first, our inability to code the videos blind because the lead researcher (NG) both performed the gentling and coded the videos. Second, age was a confounding factor in allocation to treatment, but it did not affect response to treatment. Apart from this factor, there were no significant differences in cat demographics in allocation to treatment.

5. Conclusions

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.

Conflict of interest statement

NG, SCH and CJCP have no conflicting interests with this paper's subject material. This work's sponsors played no role in this study other than financial support.

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672 Figure captions

673 Fig.1 Proportion of cats (n= 139) rated as Anxious or Frustrated over ten days at the shelter in
674 Control (CA) and Gentled groups (TA). Days 1 and 2 (N = 139 cats; CA: N=69, TA: N=70), Day
675 3(N= 133 cats, CA: N=63, TA: N=67), Day 4 (N = 130 cats CA:N=60, TA: N=63), Day 5 (N=
676 128 cats, CA N=58, TA N= 62), Day 6 (N= 127 cats, CA N=57, TA N=61), Day 7 (N = 123 cats,
677 CA N=53, TA N=60), Day 8 (N = 119 cats, CA N=49, TA N=58), Day 9 (N= 118 cats CA
678 N=48, TA N=57) , Day 10 (N= 1115 cats, CA: N=45, TA: N=57)

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680 Fig.2 Secretory immunoglobulin A (± SE) over days for Gentled (n = 70) and Control (n =69)
681 cats

682 Fig.3: Cumulative probability of onset of clinical URD over time in for Gentled (n = 70) and
683 Control (n = 69) cats.

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687 Fig.1: Proportion of cats (N=139; ± SD) rated as Anxious or Frustrated over ten days at the
688 shelter in Control and Gentled groups

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690 Fig.2: Change in mean s-IgA (± SD) over days for Gentled and Control groups.

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692 Fig.3: Cumulative probability of onset of clinical URD over time in for Gentled (n = 70) and
693 Control (n = 69) cats.

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Table 1: Behaviors (and their definitions) required to be present (*and absent*) for the rating of mood

Rating	Behavior observed > 80% per 24 hour
Anxious	
Hide	Hides under bedding, behind or in litter box
Freeze	Remains completely immobile, body and head flattened to floor
Flat	Low body posture when lying down, sitting or standing for locomotion
Startle	Sudden retreat to back of cage
Sit front*	May sit at the front of the cage but only when humans are not in the room
Absent	*All Contentment behaviors*
Frustrated	Behavior observed >10 % of awake time
Escape bouts	Very active: on hind limbs, pawing wall, paw through door, push on door latch, hanging on cage door with body inverted, scans all parts of the cage, pacing at the door. May bite cage bars*.
Meow	Regular meow, not related to anticipation of food
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.
Absent	*All Anxious behaviors and lie on side*
Content	Behavior observed > 80% per 24 hour
Lie on side	When sleeping or resting, body and tail stretched, neck and ventral area exposed.
Tall	High body posture, head held high when resting, standing and walking
Sit front	Sits at the front of the cage, calmly looking around
Rub	Rubs body or head on objects and cage door
Absent	*All Anxious behaviors*

*bar biting was not included in the original study (Gourkow et al., 2014) but was added to this study as it was frequently observed.

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706 Table 2: Criteria used for rating of responses to Gentling as negative or positive

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Response type	Description of responses
Negative	Defensive aggression: onset of hissing, growling with or without paw strike Defensive retreat: flattens body and ears, freezes or retreats
Positive	Calm: relax body, lowers head when petted between ears, raises head when petted on chin. Absence of defensive aggression or retreat 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

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

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	Control		Gentled		
	N	%	N	%	P-value
Adult	42	60.87	28	40.00	
Juvenile	8	11.59	13	18.57	0.05
Senior	19	27.54	29	41.43	
Intact	22	31.88	24	34.29	0.09
Neutered	47	68.12	46	65.71	
Male	36	52.17	47	67.14	0.10
Female	33	47.83	23	32.86	
Owner-surrendered	41	59.42	50	71.43	0.19
Stray	28	40.58	20	28.57	
Total	69	49.6	70	50.4	

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721 Table 4: Difference in Mood for cats that were owner-surrendered or stray.

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Source	N days	Mood Rating	N	%
Owner surrendered	359	Anxious	214	60
		Content	138	38
		Frustrated	7	2
Stray	206	Anxious	119	58
		Content	64	31
		Frustrated	23	11

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727 Table 5: Positive and negative responses to Gentling treatment according to
728 their daily mood classification of Content, Anxious or Frustrated (P<0.0001)
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	Negative		Positive		
Mood Rating (n days)	N	%	N	%	p-value
Content (315 days)	43	14	272	86	
Anxious (226 days)	73	32	153	68	
Frustrated (22 days)	16	73	6	27	<0.0001

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733 Table 6: Mean S-IgA concentrations in faeces for Control and Gentled cats that responded either
734 positively or negatively to gentling.
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S-IgA, $\log_e$ µg/g			
Groups	Mean	SD	p-value
Control	5.9	0.80	
Gentled	6.9	0.77	< 0.0001
Responses			
Negative	6.14	0.821	
Positive	7.03	0.608	<0.0001

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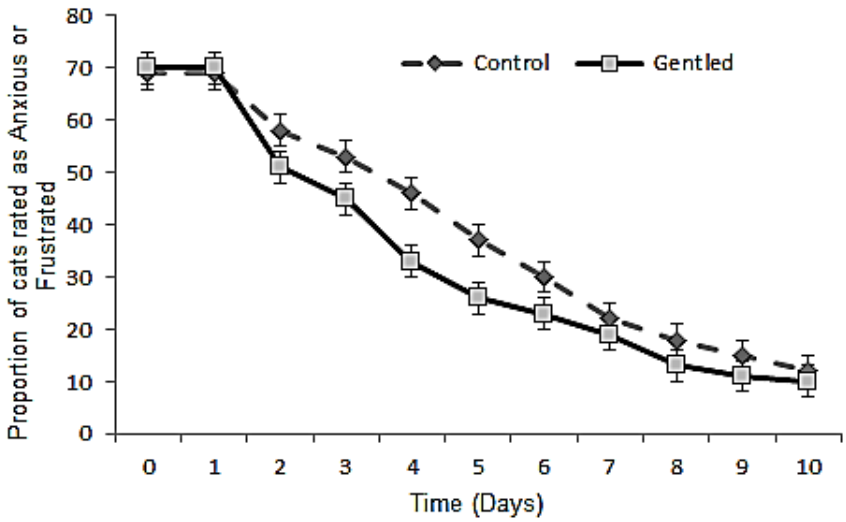

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739 Fig.1: Proportion of cats (N=139; ± SD) rated as Anxious or Frustrated over ten days at the
740 shelter in Control and Gentled groups

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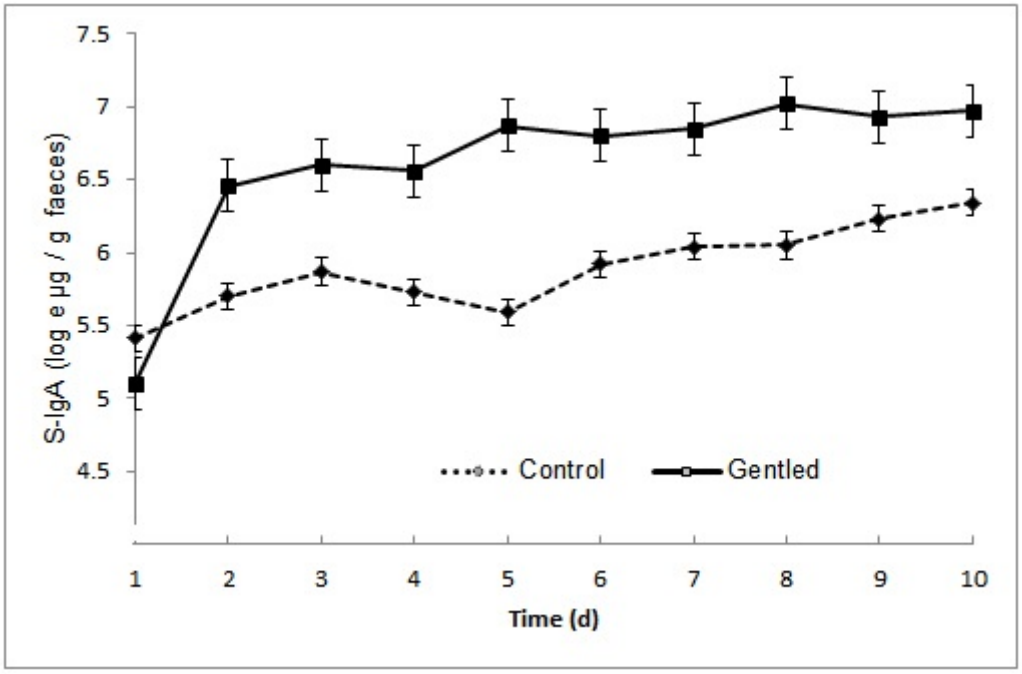

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745 Fig.2: Change in mean s-IgA (± SD) over days for Gentled and Control groups.

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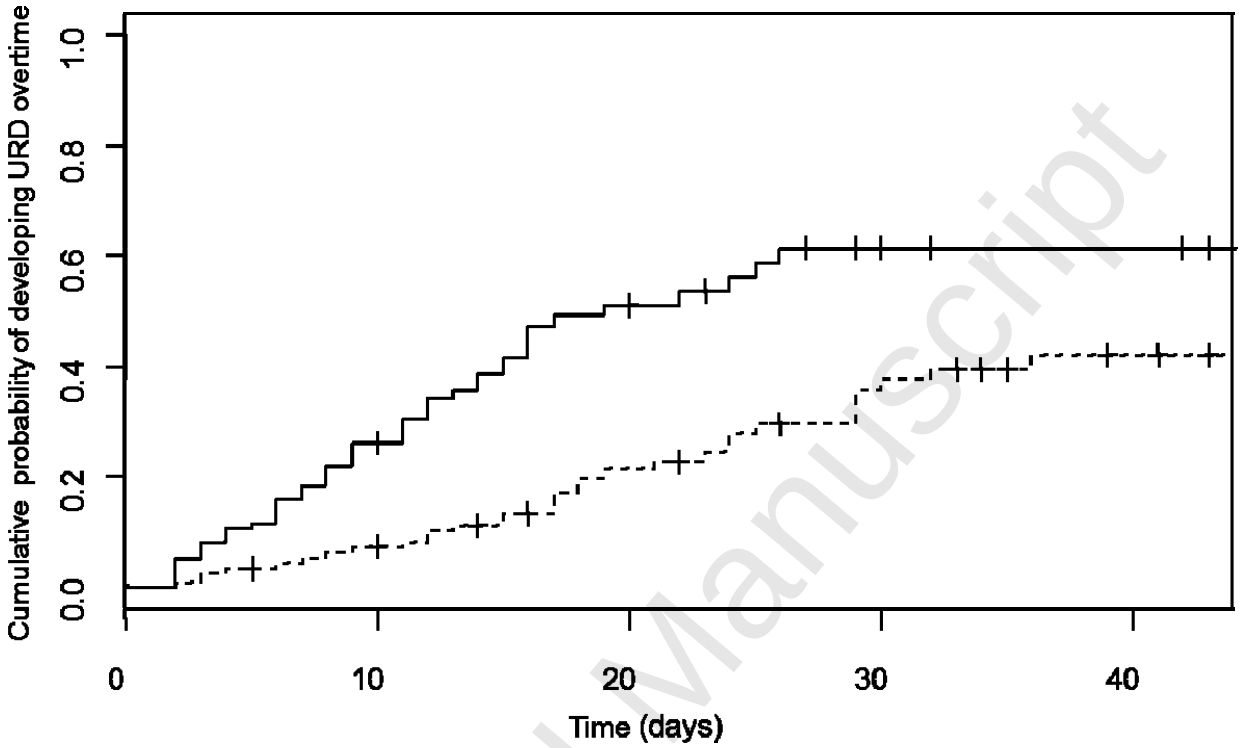

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749 Fig.3: Cumulative probability of onset of clinical URD over time in for Gentled (n = 70) and
750 Control (n = 69) cats.

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754 Highlights

755 We selected cats that were anxious upon admission to a shelter

756 One half of the cats rated as anxious were gentled with stroking and vocalisations

757 Gentled cats were more contented and less anxious/frustrated than Control cats

758 Gentled cats had improved immune status, with increased secretory IgA

759 Gentled cats had lower pathogen shedding rates and less upper respiratory disease

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