Effect of a synthetic feline facial pheromone product on stress scores and incidence of upper respiratory tract infection in shelter cats

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OBJECTIVE
To determine whether a synthetic feline facial pheromone product would decrease stress scores and upper respiratory tract infection (URI) incidence in shelter-housed cats.

DESIGN
Randomized controlled clinical trial.

ANIMALS
336 stray, feral, owner-relinquished, or legally impounded cats at 2 animal shelters in northern California.

PROCEDURES
5 cat holding rooms (3 at shelter A and 2 at shelter B) were used. A diffuser containing either synthetic pheromone or placebo was randomly assigned to each room, and cats were exposed for a 21-day period. Data collected on each cat included signalment, daily stress scores, and daily URI incidence. After 21 days, diffusers were removed for a 7-day washout period. The type of diffuser in each room was switched, and data were collected for another 21 days. Findings were statistically compared between exposure types and other groupings.

RESULTS
Cox proportional hazard analysis revealed no significant difference between exposure (pheromone or placebo) and URI incidence. Mixed-effects ordinal logistic regression revealed no significant relationship between exposure and daily stress scores. Three covariates had significant ORs: number of days in holding (OR, 0.80; 95% confidence interval [CI], 0.76 to 0.84), owner-relinquished versus stray (OR, 3.25; 95% CI, 1.18 to 8.94), and feral versus adult cat room at shelter A (OR, 11.10; 95% CI, 4.47 to 27.60).

CONCLUSIONS AND CLINICAL RELEVANCE
No evidence was found that the evaluated synthetic feline facial pheromone product had any effect on stress scores or URI incidence in shelter-housed cats. Therefore, other established methods for stress and URI reduction should be used in shelter settings. (*J Am Vet Med Assoc* 2017;251:413–420)

The American Society for the Prevention of Cruelty to Animals estimates that 1.4 million cats are euthanized annually in US shelters because of acute or chronic disease, undesirable behavior, prolonged duration of stay, and shelter overcrowding.[1] Upper respiratory tract infection is the most common disease affecting shelter- and group-housed cats and is therefore an important welfare concern.[2–4] Such infections cause considerable illness and can be fatal even with treatment, particularly in kittens or severely infected or immunocompromised cats. Some shelters have the capacity to treat sick cats and ultimately place them up for adoption, but others are forced to euthanize ill cats because of financial constraints, lack of personnel, or lack of quarantine space.[2,4,5]

In shelter cats, URI is a multifactorial disease; risk factors include inadequate sanitation, high population density, exposure to infectious cats, inadequate ventilation, young age, prolonged shelter stay, vaccination status, and stress.[2,5,6] Shelters in which cat housing or shelter protocols are adapted to address these risk factors can have substantially lower URI rates.[2,6] One of the most powerful and cost-effective ways of reducing the incidence of URI is through stress reduction, which can be achieved by making physical changes to the shelter design, enlarging cage size, reducing cat density, and adding cat socialization and behavioral enrichment programs.[2,4,6–8]

In recent years, pheromone treatment has become popular in shelters for stress reduction ow-

ABBREVIATIONS

CI	Confidence interval
URI	Upper respiratory tract infection

ing to its fairly low cost and easy implementation. However, there are no published reports that it is effective in shelter-housed cats.[9,10] Despite this, the authors of several prominent shelter and veterinary publications recommend that shelter personnel use pheromone treatment on an ongoing basis in their cat housing.[8,11–13] Therefore, there is a need to investigate the effectiveness of pheromone treatment in a shelter environment.

A synthetic feline facial pheromone has been manufactured since the 1990s and is marketed as a means to decrease urine marking and other stress-related behaviors of cats in the home.[14] Numerous studies of its effectiveness have been performed, mostly in homes of owned cats with inappropriate urination or urine marking, although a few studies have also been conducted in veterinary hospitals. Positive findings vary from slightly beneficial outcomes to dramatic decreases in frequencies of stress-related behaviors.[13,15–18] In contrast, other studies[19–21,a] have revealed no effect or ambiguous results. No adverse effects have been associated with this product. To date, the effect of this pheromone on signs of stress in cats housed in a shelter environment has not been evaluated.

The purpose of the study reported here was to determine whether diffuser administration of a synthetic feline facial pheromone product would be effective at decreasing signs of stress and incidence of URI in shelter cats. If so, this pheromone treatment could be a cost-effective and easy method for improving cat health and welfare and ultimately decreasing cat mortality rate in shelters. If not, shelter personnel should be aware of the limitations of this treatment, and resources should be focused on other proven methods of stress reduction and URI prevention.

Materials and Methods

Ethics statement

The study protocol was reviewed and approved by the University of California-Davis Institutional Animal Care and Use Committee. Permission for participation of shelter cats was obtained from the managing veterinarians at each shelter.

Cats

The study was performed at 2 animal shelters (A and B) in northern California from March through May 2015. All cats housed in 5 holding rooms (3 in shelter A and 2 in shelter B) for a minimum of 24 hours were enrolled in the study, unless they had signs of URI on day 0 (first day of diffuser exposure) or had been previously enrolled. This represented an open population for study purposes, with each enrolled cat observed and contributing time to the study for as long as it remained in the holding room (range, 24 hours to 21 days), per shelter standard protocol. Each cat was assigned a unique shelter identification number.

Shelters

Shelter A was an open-admission municipal shelter with an annual intake of approximately 4,000 cats and a live release rate of approximately 60%. Shelter B is a private, nonprofit, open-admission shelter with an annual intake of approximately 5,500 cats and a live release rate of approximately 70%. Cat admissions at shelter A included stray cats, feral cats, and animals impounded by humane control officers. Owners who wished to relinquish their pet cats were instructed to take them to shelter B. Cat admissions at shelter B included mainly owner-relinquished cats, some stray cats and kittens, and animals impounded by humane control officers. At both shelters, feral cats were admitted for trap-neuter-vaccination-return and the public was discouraged from bringing in feral cats for euthanasia.

The 3 cat holding rooms at shelter A included 1 designated for nursing queens and kittens < 1 year of age (shelter A kitten room), another for adult stray cats that would be made available for adoption (shelter A adult cat room), and a third for feral cats awaiting gonadectomy or return to their colony (shelter A feral cat room). The 2 cat holding rooms at shelter B included a room for nursing queens and kittens < 1 year of age (shelter B kitten room) and a room for adult cats ≥ 1 year of age (shelter B adult cat room). Adult feral cats were returned to colony managers the same day as gonadectomy, so no adult feral cats were housed at this shelter. Although shelter personnel attempted to put cats in the appropriate holding room per shelter protocols, this was not always possible during the study because of space constraints.

At shelter A, all cats in the holding rooms were housed in stainless steel cages of various sizes. All cages in the kitten room and feral cat room and 12 of the 30 cages in the adult cat room had 1 compartment. The cages in the kitten and adult cat holding areas had an elevated resting area, built-in shelf, or raised bed. If a feral cat was housed in a cage, the feral-cat box (a small, enclosed, hard plastic carrier with a removable plastic front panel) was left in the cage for safety of personnel, to provide a hiding area, and as a method of transportation. The remaining 18 cages in the adult cat room had 2 compartments, the smaller of which was bilevel and accessible through portals and a shelf in the large compartment. Cats were provided with a towel, food, water, and a litter box. Cages were spot cleaned by shelter personnel and completely cleaned between occupants. Ventilation was provided in each room, but the number of air exchanges per hour was unknown by shelter personnel.

At shelter B, all cats and kittens in the holding rooms were housed in identical hard-plastic cages. All of the cages had a built-in shelf and a portal leading into a smaller single level compartment where the litter box was kept. Many of the cages also contained an additional portal into the adjoining cage that was opened to provide extra space for litters of kittens or multiple cats from the same household. Cats were

provided with bedding, food, water, a litter box, and at least 2 toys. Cages were spot cleaned by shelter personnel and completely cleaned between occupants. No continuous ventilation system was present in these holding rooms.

Interventions

Each diffuser type was identified with an arbitrarily assigned code (A or B) to facilitate data collection and observer blinding. Each holding room was randomly assigned a diffuser by use of the random number generator in an electronic spreadsheet,[b] with odd numbers assigned diffuser A and even numbers assigned diffuser B. All diffusers,[c] purchased through a commercial website, were comprised of 2 components: a bottle containing the pheromone product and a diffusion device into which the bottle was inserted. To promote dispersion of pheromone into the environment, the diffusion device containing the bottle was plugged into an electrical outlet. The original bottle of synthetic feline facial pheromone that came with the diffusion device was used for the pheromone diffusers. To prepare the placebo diffusers, original bottles containing the pheromone product were used. The plug on each bottle was removed, and the wick extending into the interior of the bottle was broken off. The pheromone product was poured out, and the inside of the bottle was cleaned with soap and water. Bottles were allowed to dry, then mineral oil (the inactive base of the pheromone product) was instilled and the plug and diffusion device were replaced. Pheromone and placebo bottles were covered with identical opaque duct tape to prevent visual identification of placebo bottles. One diffuser was plugged into a functioning and unobstructed wall outlet in each holding room, as assigned. Only 1 diffuser/room was deemed necessary per the manufacturer's recommendations because all study rooms were ≤ 500 to 700 feet^2 in area.[14]

Data collection began 24 hours after diffusers were initially plugged into outlets (ie, on day 1) to allow sufficient product dispersion and to ensure the diffusers were functional. Diffusers were identified as functional when the coils within the diffusion device heated the top of the inserted bottle to the touch. Data collection continued every 24 hours for 21 consecutive days. This period was selected because the manufacturer recommends replacing the pheromone-containing bottle monthly.[14] After 21 days, the diffusers were removed for 7 days to allow washout of treatments from the room. The pheromone-containing bottles were replaced with commercially purchased product refill bottles, and the placebo diffuser bottles were refilled with mineral oil. Then the opposite type of diffuser was plugged into the outlet in each holding room for another 21-day cycle of data collection. In other words, exposures were switched between the first and second sets of data collection, so that rooms into which pheromone was previously diffused had placebo diffused instead and vice versa.

Data collection

One investigator (RMC), who was blinded to diffuser type, performed data collection for the duration of the study. This investigator had extensive experience with animal behavior and was trained to interpret cat body language and assign stress scores. Data collected included diffuser code and location (shelter and room); distance from cage to diffuser (< 5 feet, 5 to 10 feet, 10 to 15 feet, 15 to 20 feet, and 20 to 25 feet); cat source (stray, feral, owner-relinquished, or confiscated), sex, estimated age, and reproductive status (neutered or sexually intact) at intake; number of days the cat was in the holding room; and number of cats sharing the cage.

The blinded investigator evaluated each cat and assigned stress and URI scores on a daily basis. The stress score system of Kessler and Turner was used as described in detail elsewhere[22] to characterize the overall stress level of each cat by observed body postures (body, belly, limbs, and tail), degree of eye opening, degree of pupillary dilation, ear and whisker position, vocalization, and activity as follows: 1 = fully relaxed, 2 = weakly relaxed, 3 = weakly tense, 4 = very tense, 5 = fearful, 6 = very fearful, and 7 = terrorized. Cats were evaluated for clinical signs of URI on the basis of mucoid or purulent ocular or nasal discharge and active sneezing.[23] If cats were in the medical clinic or hiding from the investigator's view, values were listed as unknown for that day, but this did not disqualify cats from being scored on subsequent days.

Statistical analysis

All data were recorded in electronic spreadsheets.[c] Statistical analyses were performed by use of statistical software.[d] For each statistical test, assumptions were checked. Data were initially summarized by calculation of descriptive statistics. Distributions of stress scores per day for each location (holding room) were assessed visually by construction of normal probability plots, revealing a nonnormal distribution.

Cox proportional hazard analysis was performed to compare the incidence of URI between diffuser types (pheromone vs placebo), with time to failure defined as the time (in days) from day 0 until a cat developed signs of URI. The proportional hazards assumption was tested.[d] An initial model was created that included the independent variables diffuser type; location; cat source, age, and sex; distance from cage to diffuser; and number of cats in the cage. The interaction between location and diffuser type was then added to the model, revealing a significant ($P < 0.05$) association with URI development. Consequently, subsequent models were stratified by location.

For each location, a model was created that included the main effects diffuser type; location; cat source, age, and sex; distance from cage to diffuser; and number of cats in the cage. Locations that lacked a sufficient number of incident URI cases (ie, ≤ 5% of cats with URI identified) were excluded from further

analysis. For each location, main effect variables represented by an insufficient number of cats to allow model convergence were also excluded from the model. Location models were initially analyzed without any interactions, and then augmented with interaction terms involving diffuser type and each of the other main effects in turn. Significant interactions were included in the final models for each location. The same approach was used with cat source as the unit of stratification, and models were so created for stray cats and owner-relinquished cats. Given the small incidence of URI, post hoc power calculations were completed by use of an online power calculator.[e]

Mixed-effects ordinal logistic regression was used to compare daily stress scores (1 to 7, with no collapsing of categories) between diffuser types. The variables considered in the full model were number of days spent in the holding room; diffuser type; lo-

Table 1—Characteristics of cats (n = 336) exposed to a diffuser containing a synthetic feline facial pheromone product or placebo (mineral oil) for up to 21 days in 5 holding rooms at 2 northern California animal shelters.

	Shelter A			Shelter B	
Variable	**Adult (n = 68)**	**Feral (n = 91)**	**Kitten (n = 14)**	**Adult (n = 112)**	**Kitten (n = 51)**
Exposure					
Placebo	38 (56)	36 (40)	4 (29)	53 (47)	29 (57)
Pheromone	30 (44)	55 (60)	10 (71)	59 (53)	22 (43)
Distance from cage to diffuser (feet)					
< 5	0 (0)	11 (12)	7 (50)	0 (0)	0 (0)
5–10	33 (49)	33 (36)	2 (14)	22 (20)	0 (0)
10–15	25 (37)	20 (22)	5 (36)	20 (18)	1 (2)
15–20	10 (15)	27 (30)	0 (0)	62 (55)	47 (92)
20–25	0 (0)	0 (0)	0 (0)	8 (7)	3 (6)
Source					
Stray	58 (85)	87 (96)	9 (64)	7 (6)	39 (76)
Relinquished	8 (12)	4 (4)	0 (0)	105 (94)	12 (24)
Confiscated	2 (3)	0 (0)	5 (36)	0 (0)	0 (0)
Age (y)					
< 1	4 (6)	3 (3)	9 (64)	6 (5)	41 (22)
1–8	51 (75)	85 (93)	5 (36)	79 (71)	10 (20)
> 8	13 (19)	3 (3)	0 (0)	27 (24)	0 (0)
Sex					
Male	28 (41)	33 (36)	3 (21)	50 (45)	22 (43)
Female	39 (57)	43 (47)	11 (79)	62 (55)	25 (49)
Unknown	1 (2)	15 (16)	0 (0)	0 (0)	4 (8)
Number of cats in the cage					
1	68 (100)	89 (98)	3 (21)	96 (86)	19 (37)
2	0 (0)	2 (2)	6 (43)	16 (14)	12 (24)
3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
4	0 (0)	0 (0)	0 (0)	0 (0)	4 (8)
5	0 (0)	0 (0)	5 (36)	0 (0)	10 (20)
6	0 (0)	0 (0)	0 (0)	0 (0)	6 (12)
Number of days spent in the holding room	3 (1–11)	4 (1–20)	4 (1–12)	4 (1–18)	3 (1–8)
URI incidence	9 (13)	18 (20)	0 (0)	4 (4)	3 (6)
Number of days to URI development	3 (1–7)	4 (1–8)	—	3.5 (2–7)	1 (1–2)
Stress score on day 1					
Placebo exposure	3 (1–6)	4 (2–5)	2 (2–4)	3 (1–5)	3 (2–4)
Pheromone exposure	3 (2–5)	4 (1–5)	3 (2–4)	4 (1–5)	3 (2–5)

Data involving stress scores and number of days are reported as median (range). All other values are reported as number (%) of cats in the group to which the indicated variable pertains.

— = No cats in this group

This was an open population, with 336 cats enrolled over the entire study period. A pheromone or placebo diffuser was placed in each of 5 holding rooms (3 in shelter A [for kittens, adoptable stray adult cats, and feral cats] and 2 in shelter B [for kittens and adult cats]) for a 21-day period. Diffusers were then removed, and after a 7-day washout period, the opposite diffuser was placed in the rooms for an additional 21-day period. Each cat contributed a minimum of 24 hours and maximum of 21 days of observations.

cation; cat source, age, and sex; distance from cage to diffuser; and number of cats in the cage. Diffuser type, day, and location were included in a main effects–only multivariable model, and interactions between these variables were tested for improvement in fit in subsequent multivariable models. Significant ($P < 0.05$) interactions were included in the final model.

Results

Animals

A total of 336 cats met inclusion criteria and were enrolled in the study **(Table 1)**. The amount of time each cat contributed to the study varied. Five of the cats in the kitten holding room in shelter A were adults. These cats had been confiscated from a home and remained in the shelter for > 1 week, whereas the other 9 kittens were feral and sent to foster care within 1 or 2 days after shelter admission. For these reasons, all data related to this location were excluded from statistical comparisons, resulting in inclusion of only 322 of the original 336 (96%) cats.

Thirty-four (11%) cats developed signs of URI over the observation period; 1,526 daily stress scores were recorded. Ten cats exposed to the placebo diffuser had unknown stress scores on day 1, 39 on day 2, and 3 on day 3. Four cats exposed to the pheromone diffuser had unknown stress scores on day 1, 64 on day 2, and 7 on day 4.

Table 2—Results of Cox proportional hazard analysis of the incidence of URI in the cats exposed to a diffuser containing a synthetic feline facial pheromone product or placebo (mineral oil) for up to 21 days in the adoptable stray adult (n = 68) and feral (91) cat rooms at shelter A.

Variable, by holding room	Hazard ratio (95% CI)	*P* value
Adult cat room		
Diffuser		
Placebo	Referent	—
Pheromone	0.53 (0.10–2.78)	0.46
Distance from cage to diffuser (feet)		
< 5	Referent	
5–10	NC	
10–15	0.44 (0.075–2.60)	0.37
15–20	3.09 (0.46–20.8)	0.25
20–25	NC	
Age (y)		
< 1	Referent	—
1–8	0.16 (0.02–1.17)	0.07
> 8	0.11 (0.01–1.59)	0.11
Sex		
Male	Referent	—
Female	0.53 (0.12–2.34)	0.40
Unknown	NC	—
Feral cat room		
Diffuser		
Placebo	Referent	—
Pheromone	0.93 (0.36–2.40)	0.87
Distance from cage to diffuser (feet)		
< 5	Referent	—
5–10	7.12 (0.77–66.1)	0.08
10–15	1.94 (0.15–25.5)	0.61
15–20	3.59 (0.39–32.8)	0.26
20–25	NC	—
Age (y)		
< 1	Referent	—
1–8	0.59 (0.12–2.87)	0.51
> 8	1.25 (0.14–11.1)	0.84
Sex		
Male	Referent	—
Female	0.32 (0.10–1.02)	0.053
Unknown	0.40 (0.06–2.44)	0.32
Source		
Stray	Referent	—
Relinquished	6.14 (0.50–75.90)	0.16
Confiscated	NC	—

— = Not applicable. NC = Not calculated because data did not converge.
See Table 1 for remainder of key.

Table 3—Results of Cox proportional hazard analysis of the incidence of URI in the cats represented in Table 1 (n = 322), by cat source.

Variable, by cat source	Hazard ratio (95% CI)	*P* value
Stray		
Diffuser		
Placebo	Referent	—
Pheromone	0.68 (0.31–1.49)	0.33
Distance from cage to diffuser (feet)		
< 5	Referent	—
5–10	2.41 (0.28–21.10)	0.43
10–15	1.20 (0.12–12.30)	0.88
15–20	3.47 (0.43–28.30)	0.25
20–25	NC	—
Age (y)		
< 1	Referent	—
1–8	0.74 (0.15–3.64)	0.15
> 8	0.80 (0.11–5.63)	0.11
Sex		
Male	Referent	—
Female	0.50 (0.22–1.13)	0.10
Unknown	0.96 (0.17–5.32)	0.17
No. of cats in the cage		
1	Referent	—
2	7.18 (1.02–50.70)	0.048
3	NC	—
4	1.88 (0.08–44.30)	0.70
5	NC	—
6	NC	—
Location*		
Shelter A adult cat room	Referent	—
Shelter A feral cat room	0.66 (0.24–1.86)	0.43
Shelter B adult cat room	0.40 (0.04–3.61)	0.41
Shelter B kitten room	0.21 (0.02–1.84)	0.16
Owner relinquished		
Diffuser		
Placebo	Referent	—
Pheromone	3.19 (0.32–31.50)	0.32
Sex		
Male	Referent	—
Female	2.48 (0.24–25.80)	0.45
Unknown	NC	—
No. of cats in the cage		
1	Referent	
2	1.16 (0.11–12.30)	0.90
3	NC	—
4	NC	—
5	NC	—
6	NC	—

*Cats in the kitten holding room at shelter A were omitted from this analysis because of the small sample size and deviations from study protocol.
See Tables 1 and 2 for remainder of key.

URI

Respective incidences of URI for placebo- and pheromone-exposed cats by holding room were as follows: shelter A cat room, 18% (7/38) and 7% (2/30); shelter A feral cat room, 22% (8/36) and 18% (10/55); shelter B adult cat room, 2% (1/53) and 5% (3/59); and shelter B kitten room, 3% (1/29) and 9% (2/22). Cox proportional hazard analysis revealed no significant difference in URI incidence between placebo- and pheromone-exposed cats. With data stratified by location (shelter and holding room), no variables were identified as significantly associated with URI development in the cats of the adult (URI incidence, 13%) and feral (URI incidence, 20%) cat holding rooms at shelter A **(Table 2)**. Data for the adult cat and kitten holding rooms of shelter B could not be modeled because of sparse data.

With data stratified by cat source (excluding confiscated cats, for which data were too sparse to permit modeling), stray cats housed 2/cage was the only variable significantly (P = 0.048) associated with URI development (hazard ratio, 7.18; 95% CI, 1.02 to 50.70; **Table 3**). Statistical power in all incidence comparisons (determined post hoc) ranged from 5% to 21%.

Table 4—Results of mixed-effects ordinal logistic regression of daily stress scores for the cats in Table 1 (n = 322).

Variable	OR (95% CI)	*P* value
No. of days spent in holding room	0.80 (0.76–0.84)	< 0.001
Diffuser		
Placebo	Referent	—
Pheromone	0.92 (0.52–1.63)	0.77
Cat source		
Stray	Referent	—
Owner relinquished	3.25 (1.18–8.94)	0.02
Confiscated	2.31 (0.06–81.9)	0.65
Location*		
Shelter A adult cat room	Referent	
Shelter A feral cat room	11.10 (4.47–27.60)	< 0.001
Shelter B adult cat room	0.51 (0.16–1.64)	0.26
Shelter B kitten room	0.30 (0.07–1.38)	0.12
Age (y)		
< 1	Referent	
1–8	0.69 (0.20–2.31)	0.54
> 8	0.26 (0.62–1.05)	0.06
Sex		
Male	Referent	—
Female	1.09 (0.60–1.97)	0.78
Unknown	0.74 (0.16–3.47)	0.70
Distance from cage to diffuser (feet)		
< 5	Referent	—
5–10	0.80 (0.15–4.29)	0.79
10–15	0.86 (0.15–4.83)	0.87
15–20	1.16 (0.21–6.28)	0.87
20–25	1.30 (0.14–11.90)	0.82
No, of cats in the cage		
1	Referent	—
2	0.86 (0.29–2.58)	0.79
3	NC	NC
4	NC	NC
5	2.19 (0.35–13.70)	0.40
6	0.26 (0.02–2.80)	0.27

The stress score system of Kessler and Turner was used as described in detail elsewhere[23] to characterize the overall stress level of each cat by observed body postures (body, belly, limbs, and tail), degree of eye opening, degree of pupillary dilation, ear and whisker position, vocalization, and activity as follows: 1 = fully relaxed, 2 = weakly relaxed, 3 = weakly tense, 4 = very tense, 5 = fearful, 6 = very fearful, and 7 = terrorized.

See Tables 1 and 2 for remainder of key.

Stress scores

Mixed-effects ordinal logistic regression revealed no significant association between diffuser type and daily stress scores **(Table 4)**. Three covariates had a significant OR in the final model: number of days spent in the holding room (OR, 0.80; 95% CI, 0.76 to 0.84; $P < 0.001$), owner-relinquished versus stray cats (OR, 3.25; 95% CI, 1.18 to 8.94; $P = 0.02$), and the feral cat versus adult cat holding rooms in shelter A (OR, 11.10; 95% CI, 4.47 to 27.60; $P < 0.001$).

Discussion

The incidence of URI in cats during the holding period at the 2 animal shelters in the present study was fairly low. By stratifying the data by location (shelter and holding room) and cat source, it was possible to examine differences between groups in daily stress scores and URI incidence and any effect of the diffuser-administered synthetic feline facial pheromone product on these outcomes within groups. Despite the many analyses performed, no significant effect of the pheromone diffuser was identified. Stray cats housed in groups of 2 had 7 times the hazard of stray cats housed singly for developing URI during the holding period, affirming that stray cats should be housed separately and not placed in shared cages even when space is restricted. The incidence of URI was highest in the feral cat room (20%) and adult cat room (13%) of shelter A. Shelter B had such a low incidence of URI that proportional hazards analysis could not be performed. This low incidence may have been attributable to several factors, including cage type and size, cat sociability, room cleaning methods and products, vaccination protocols, or noise level.

The daily stress score analysis also revealed no significant difference between cats exposed to the pheromone diffuser and those exposed to the placebo diffuser. The OR was significant for 3 other covariates. Higher stress scores were observed in owner-relinquished cats versus stray cats, and in cats held in the feral cat room versus the adult cat room in shelter A, and a decrease in daily stress scores was observed with number of days spent in the holding room. Other researchers have also shown that shelter cats have high stress scores on admission, which decline over time.[6] No difference between diffuser types was found in the day 1 median stress scores or the rate of stress score decline in the present study. As would be expected, feral cats had substantially higher stress scores than adult stray cats considered to be highly adoptable, which likely reflects their lack of socialization. The OR for stress scores of owner-relinquished versus stray cats (3.25) may have indicated that stray cats were better able to adapt to a new environment than previously owned cats.

One precaution when interpreting the results of the study reported here arises from the intrinsic differences among shelters in the types of cats admitted and housing provided. For example, shelter A almost exclusively admitted stray or feral cats, whereas shel-

ter B admitted mostly owner-relinquished adult cats and stray kittens. All of the cats in shelter B had double-compartment housing, whereas only some of the cats in shelter A had similar housing. Ambient noise, environmental cleaning methods, interactions with personnel, and visual contact with other cats also varied between the shelters and among holding rooms, and any or all of these variables could be important sources of stress and therefore potential confounders.

Another important consideration is that shelter A had a ventilation system in each holding room, but shelter B did not. It is possible that the diffusers in shelter A were ineffective because of a high rate of air exchange that diluted a potential beneficial effect of the pheromone product. Conversely, the lack of ventilation in shelter B theoretically permitted sufficient aerosolization of pheromone within those holding rooms, therefore supporting our finding of a lack of effect. Although it is unlikely that this pheromone product would be beneficial only to stray or feral cats and ineffective for owner-relinquished adult cats and stray kittens, the difference in ventilation systems cannot be excluded as a potential confounding factor. Repeating this study with a pheromone product sprayed directly onto bedding could help clarify this point; however, the act of daily reaching into a cage to apply the product could in itself cause stress to cats, thereby underscoring the inherent difficulty in performing a perfectly controlled study in multiple shelter environments.

Although the statistical analyses in the present study revealed a highly variable and nonsignificant association between the synthetic feline facial pheromone produce and the incidence of URI during the holding period, a post hoc power analysis revealed that the statistical power of the study to find significant associations if they truly existed was low (5% to 21%). This was due in part to the fairly low incidence of URI in the study. A larger sample size would be necessary to confirm that the pheromone product was no different than a placebo in this respect. For example, to detect a 50% decrease in the incidence of URI between groups during the maximum 21-day holding period, the study would require approximately 300 cats in the shelter A adult cat room per treatment group to achieve 80% power, assuming a 13.24% probability of cats developing URI and an α value of 0.05. However, sufficient power existed in the study to detect other covariates significantly associated with URI development, so although statistical power was low, the effects of some other covariates were strong enough to be reflected in statistical analysis. Thus, the mitigating effects of other interventions are likely of substantially more importance in the prevention of URI than use of a pheromone diffuser for cats in a shelter setting.

It is important to note that the findings of the present study do not apply to the use of pheromone diffusers in the home. The specific pheromone used is a synthetic copy of the F3 feline facial pheromone; it is believed to help calm and soothe cats that are in stressful situations or in a new environment by mimicking territorial markings.[14,16,17] The general stress levels and specific triggers of cats in shelter settings are different than those of owned cats in home settings, so these pheromone diffusers may be adequate for the specific needs of owned cats but lack a strong enough effect to benefit cats that are newly admitted to a foreign shelter environment. Additionally, during data collection, a new pheromone product[f] became commercially available. This new product is a synthetic copy of the feline appeasing pheromone and is marketed as reducing tension and conflict among household cats. It remains unknown whether use of this newer product would have led to different results than those reported here. Our findings did not clearly support a beneficial effect of the tested pheromone product for decreasing stress or URI development in cats admitted to shelters, but did suggest that other factors are of greater significance. Therefore, we encourage shelter personnel to invest their limited time and resources into other, established methods of stress reduction in cats.

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The authors declare that there were no conflicts of interest.

Footnotes

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From this month's *AJVR*

Effects of storage conditions on results for quantitative and qualitative evaluation of proteins in canine urine

Marie-Laure Théron et al

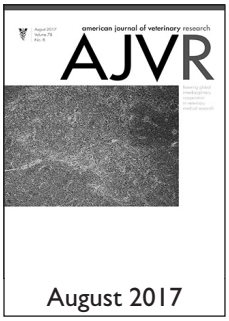
OBJECTIVE
To investigate effects of storage conditions on the canine urine protein-to-creatinine ratio (UPC) and on SDS–agarose gel electrophoresis (AGE) of urinary proteins.

SAMPLE
Urine specimens from 20 proteinuric (UPC > 0.5) and 20 nonproteinuric (UPC ≤ 0.2) dogs.

PROCEDURES
UPC and SDS-AGE were performed on urine specimens stored at room temperature (20°C) and 4°C for up to 5 days and at –20° and –80°C for up to 360 days; some specimens were subjected to 3 freeze-thaw cycles. Results were compared with those obtained for fresh urine specimens.

RESULTS
UPC was not affected by storage at room temperature or by freezing. A decrease in UPC was observed for specimens from nonproteinuric dogs after 5 days at 4°C (10%) and from both groups after 90 days at –20° and –80°C (≤ 20% and ≤ 15%, respectively). The SDS-AGE profiles revealed no visual changes regardless of duration of storage for specimens stored at room temperature, 4°C, and –80°C, except for 1 profile after 360 days at –80°C. Repeated freeze-thaw cycles did not affect SDS-AGE profiles. Appearance or strengthening of high-molecular-weight bands that could alter interpretation was evident in SDS-AGE profiles after storage at –20°C for ≥ 15 days (31/40 dogs).

CONCLUSIONS AND CLINICAL RELEVANCE
Storage of urine at –20° or –80°C for up to 1 year influenced the UPC without affecting clinical interpretation. Storage of urine specimens at –20°C impaired visual analysis of SDS-AGE. When SDS-AGE cannot be performed on fresh or recently refrigerated urine specimens, storage at –80°C is recommended. (*Am J Vet Res* 2017;78:990–999)



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