Effects of repeated petting sessions on leukocyte counts, intestinal parasite prevalence, and plasma cortisol concentration of dogs housed in a county animal shelter

Emily S. Dudley, DVM, MS; Patricia A. Schiml, PhD; Michael B. Hennessy, PhD

Objective—To describe changes in WBC counts, plasma cortisol concentration, and fecal parasite shedding of dogs housed in an animal shelter and determine the effects of daily petting sessions on these variables.

Design—Hybrid prospective observational and experimental study.

Animals—92 healthy dogs newly arrived to an animal shelter and 15 healthy privately owned dogs (control group).

Procedures—Blood and fecal samples were collected from shelter dogs 1, 3, and 10 days after arrival and from control dogs once. A subset of shelter dogs (n = 15) was assigned to receive 30 minutes of petting daily. Plasma cortisol concentration was measured, CBCs were performed, and fecal samples were evaluated for parasite ova.

Results—For shelter dogs, total leukocyte, neutrophil, and lymphocyte counts increased significantly between days 1 and 10, with less consistent increases in monocyte count and neutrophil-to-lymphocyte count ratio. Parasite shedding was unaffected by duration of shelter stay but was greater for shelter versus control dogs. For shelter dogs, plasma cortisol concentration decreased with time and was higher than that of control dogs on each day. Total leukocyte, neutrophil, and monocyte counts and neutrophil-to-lymphocyte count ratios were also higher for shelter versus control dogs. Petting sessions resulted in a decrease in plasma cortisol concentration but in no other variables.

Conclusions and Clinical Relevance—Large increasing immunologic responses, heavy parasite shedding, and high but decreasing plasma cortisol concentration were identified in shelter dogs. Daily 30-minute petting sessions affected only cortisol values, so the clinical importance of petting for immunologic and other health outcomes remains unclear. (*J Am Vet Med Assoc* 2015;247:1289–1298)

An estimated 7.6 million dogs and cats arrive at animal shelters annually in the United States and stay for variable durations.[1] Of those animals arriving at shelters, approximately 2.7 million are euthanized annually, considerable numbers of which have behavioral or physical health deficiencies.[1,2] The prevalence of intestinal parasites in dogs housed in animal shelters is reportedly between 36% and 50%, and upper respiratory infections and gastrointestinal parasites are among the most common conditions reported for dogs 1 week after adoption from a shelter.[3,4] Upper respiratory tract infections and gastrointestinal diseases may be caused by various bacterial, viral, or parasitic pathogens, and all shelters have infectious disease outbreaks.[5] Shelter management procedures to minimize the extent of infection often focus on sanitation and disease communicability; however, another important aspect to consider is the effect of stress associated with the shelter environment on the ability of an animal to mount an effective immune response.

Two primary physiologic systems are activated by stressors: the SAM axis and the HPA axis. Stimulation of the SAM axis results in the release of catecholamines, primarily epinephrine, from the adrenal medulla,[6–8] whereas stimulation of the HPA axis causes a cascade of endocrine responses and ultimately glucocorticoid secretion.[7,8] The specific glucocorticoid secreted is species

Abbreviations

HPA	Hypothalamic-pituitary-adrenal
N:L	Neutrophil-to-lymphocyte count ratio
SAM	Sympathetic-adrenal-medullary

From the Departments of Laboratory Animal Resources (Dudley) and Psychology (Schiml, Hennessy), College of Science and Mathematics, Wright State University, Dayton, OH 45435.

Supported in part by the Wright State University Microbiology and Immunology Program Graduate Student Assembly and the Waltham Foundation.

This manuscript represents a thesis submitted by Dr. Dudley to the Wright State University Microbiology and Immunology Program as partial fulfillment of the requirements for a Master of Science degree.

The authors thank Mitchel Aselage, Jennifer Beasley, Sean Collins, Feras Deek, Josh Forman, Aaron Fowler, Caroline Foy, Darci Gallimore, Alexis Garybush, Samantha Hagerty, Kalia Haile, Alex Hensley, Casey Hess, Kendra Kerner, Gregory Lowery, Sandra Matthews, Colton Metzger, Matt Shiverdecker, Cassandra Tolliver, Samantha Watts, Regina Willen, Elizabeth Wise, and Neena Zwier for assistance with data collection; Bev Grunden for statistical advice; and the Montgomery County Animal Resource Center for continued accommodation of this research.

Address correspondence to Dr. Dudley (Emily.dudley@wright.edu).

dependent; dogs secrete cortisol primarily.[7] Acute physical or psychological stress, defined as lasting for minutes to hours, is often immunoprotective, enhancing innate and adaptive responses. In contrast, chronic stress, defined as stress lasting at least several hours per day for weeks or months, can suppress or dysregulate immune function and is considered immunopathologic.[9] The combined actions of epinephrine, norepinephrine, and cortisol cause changes to the relative numbers of circulating WBCs.[9,10] In dogs, these changes include increases in numbers of circulating total leukocytes and neutrophils and decreases in numbers of circulating lymphocytes. Increases in monocyte counts are less consistent.[11] Changes likely occur because of immune cell redistribution and may compromise the effector and surveillance roles of the immune system, contributing to some of the immunosuppressive effects of stress.[8,10,12–14] The health consequences of physical and psychological stress in humans and laboratory animals are well documented and include impairment of wound healing, increase in susceptibility to viral and bacterial infections, modulation of vaccination response, and reactivation of latent herpesvirus infections.[8,15–19] Examples of the wide range of stressors evaluated in humans and other animals include chronic caregiving, academic test taking, social isolation, restraint stress, and electric shock. Opportunistic parasites that only cause mild or self-limiting infections in immunocompetent hosts may more readily infect immunocompromised individuals and, when present, often have devastating consequences.[20]

While in animal shelters, dogs are exposed to multiple stressors, including confinement, noise, social instability, separation from human companions, and loss of control in an unpredictable, novel environment. Immediate behavioral responses to these stressors, such as barking and whining, are readily observable in shelter dogs, but long-term consequences may also develop, including undesirable behaviors associated with separation anxiety.[21] Interaction with humans in the shelter environment can lead to a decrease in physiologic indicators of stress, prevent immediate undesirable behaviors, and encourage sociable behavior in dogs.[22,23] In a randomized controlled clinical trial,[24] shelter dogs for which a food-filled toy and behavioral training were provided daily had a greater increase in time spent sitting or lying down, a greater increase in the amount of quiet behavior, and a greater decrease in the amount of jumping behavior, whereas dogs that did not receive this treatment had a greater increase in jumping, whining, barking, and growling. It is possible that, in addition to the training itself, the human interaction involved with that training contributed to the observed effects.

Stressors associated with shelter housing have an effect on plasma and salivary cortisol concentrations in dogs. These concentrations are reportedly highest during the first 3 days after arrival at the shelter; after 10 days, values are more comparable to those of dogs in a home environment.[25] However, the immunologic effects of stressors inherent to shelter housing have not been fully evaluated for dogs or cats. One study[26] revealed that 60% of cats developed upper respiratory tract infections while in a shelter, and cats with more behavioral signs of stress were more likely to develop infection than were other cats. To the authors' knowledge, studies of the effect of shelter housing–related stress on immune function in dogs or changes in the immune function during shelter housing have not been reported. The impact of pathogen exposure while in a shelter on immune function and response in dogs is also unknown, particularly when that exposure occurs simultaneously with the general stress response to shelter housing.

Studies[22,27,28,29] involving shelter dogs have revealed that increases in salivary and plasma cortisol concentration can be attenuated through various types of structured human interaction, including obedience training, petting, playing, and grooming. Interestingly, the passive presence of a human remaining in the kennel with a shelter dog is also effective at reducing plasma cortisol concentration.[29] In 3 of those studies,[26,28,29] dogs were provided 1 human interaction session of 20 to 45 minutes; in the remaining study,[22] dogs received a 25-minute session on 2 days. It is unknown whether the positive effects of human interaction would persist if additional petting sessions were provided during prolonged shelter housing or if dogs would become habituated to the effect. Moreover, it is unclear how petting might affect immunologic indicators of stress in shelter dogs.

The purpose of the study reported here was to assess intestinal parasite shedding status, immunologic activity, and plasma cortisol concentration in dogs during the first 10 days of housing in an animal shelter and to determine whether daily 30-minute petting sessions would affect these variables. We hypothesized that total leukocyte, neutrophil, and monocyte counts would increase; lymphocyte counts and plasma cortisol concentrations would decrease; and fecal shedding of parasites would increase during 10 days of shelter housing. We also hypothesized that repeated petting sessions over a 10-day period would result in a decrease in immune activity, cortisol, and parasite shedding values.

Materials and Methods

Animals—Two sets of dogs were enrolled in the study: shelter dogs and privately owned dogs. Shelter dogs included dogs from the Montgomery County Animal Resource Center in Dayton, Ohio, that were brought to the shelter as strays or released to the shelter by their owners between July and October 2012 and May and July 2013. Vaccinations[a] and other routine processing procedures (which did not include the administration of anthelmintics) were performed by shelter staff when dogs arrived (day 0). Study procedures began the following day (day 1). One investigator (ESD) selected all shelter dogs for the study. Dog sex and breed information were recorded at that time. Age was estimated on the basis of the presence or lack of deciduous canine teeth[30] or clinically apparent nuclear sclerosis.[31] Dogs were excluded from the study when they had signs of aggression or fear. Dogs with a suspected pregnancy or signs of infectious disease, including diarrhea or upper respiratory tract abnormalities, were not enrolled. If a dog developed clinical signs of illness during the study period, it was excluded only when routine shelter pro-

cedures indicated that treatment was necessary. Visual assessments of the health status of shelter dogs were performed daily by shelter staff. In addition, research personnel visually assessed dogs daily for general signs of illness. At the conclusion of the study, results of fecal evaluations were promptly communicated to shelter management so that appropriate treatment could be administered.

All shelter dogs were individually housed in a large intake ward for the duration of the 10-day study. Dogs were maintained in 1 of 75 size-appropriate kennel runs, most measuring 1.5 × 1.2 × 1.8 m. Kennels were arranged on either side of central aisles, with several aisles present within the ward. Individual kennels were constructed of smooth, solid surfaces on 3 sides, with the front of the kennel constructed of a metal mesh, allowing each dog visual and auditory contact with others.

Dogs privately owned by colleagues and study assistants were recruited as a control group. The same physical and behavioral exclusion criteria as used for the dogs housed at the shelter were applied during enrollment. Control dogs were maintained in their owners' homes. Wright State University's Institutional Animal Care and Use Committee reviewed and approved all study procedures. Written consent was obtained from a shelter representative and from all owners of control dogs prior to study enrollment.

Study design—The study was conducted in 2 parts. Sample sizes were determined through power analysis on the basis of available parasite prevalence and plasma cortisol data,[3,29] with power set at 80%, and were consistent with previous sample sizes used by the authors' research group.[25,28,29] In part 1, a subset (n = 40) of the shelter dogs and all control dogs were evaluated at various points for plasma cortisol concentration, CBC values, and fecal shedding of parasites. Shelter dogs were evaluated on days 1, 3, and 10 after arrival at the shelter. On each of these days, dogs were removed from their kennels and a set of blood samples was collected; a fecal sample was also obtained. Control dogs were evaluated once, with 1 fecal sample and 1 set of blood samples collected.

In part 2, a subset (n = 52) of all shelter dogs were evaluated for the effect of human interaction on plasma cortisol concentration, CBC results, and fecal shedding of parasites during the first 10 days after arrival at the shelter. Dogs were quasirandomly assigned in the order in which they were enrolled in the study to receive 30-minute sessions consisting of 1 of 3 treatments: home kennel (n = 16), novel room (16), and petting (15). Treatment sessions were provided daily, with the exception of weekends and holidays; each dog received 7 to 8 sessions. Prior to treatment, all dogs were removed from their home kennel and walked in a small enclosed courtyard for 2 to 3 minutes. Dogs in the home-kennel group were then returned to their home kennel and did not receive additional interaction. Each dog in the novel-room group was taken to a separate room in the animal shelter and placed in a kennel measuring 1.5 × 1.5 × 1.8 m with a small blanket placed on the floor. No other dogs were in this room. Study personnel left the room for 30 minutes before returning each dog to its home kennel. Dogs in the petting group were also taken to the novel room and placed in the kennel with a blanket. However, conditions for that group differed from those for the novel-room group in that a petter stayed with each dog in the novel environment. This petter encouraged each dog to lie down, spoke to it in a soothing voice, and performed a combination of petting and deep tissue massage around the head, neck, and shoulder region of the dog for 30 minutes as described elsewhere[29,32] to encourage relaxation.[33] Petters consisted of a team of 10 female research assistants who were trained in the petting technique, and dogs received petting sessions from multiple petters during the study.

Blood samples were collected from all dogs in part 2 of the study on days 1 and 10 after arrival at the shelter. On both days, a blood sample was collected for CBC and plasma cortisol measurement immediately prior to the assigned treatment session, and an additional blood sample was collected immediately after the treatment session for cortisol measurement. Two fecal samples were also collected from each dog: the first on day 1, 2, or 3 and the second on day 10.

Blood sample collection and laboratory analysis—All blood samples were collected between 1:00 PM and 5:00 PM to control for circadian fluctuations in cortisol concentration. Interval from approach to the cage to sample collection was recorded. The venipuncture site was most often a cephalic vein; however, a lateral saphenous vein or jugular vein was also occasionally used, depending on the anatomy of individual dogs and their apparent comfort with handling. Blood samples were collected via 1-inch, 22-gauge needles into 3-mL syringes. One milliliter of each sample was transferred to a standard EDTA-containing tube, and another 1 mL was transferred into a heparin-containing tube. All samples were kept on ice until returned to the laboratory for processing.

Once blood samples arrived at the laboratory, heparinized samples were centrifuged at approximately 500 × *g* for 20 minutes, and then plasma was removed and frozen for cortisol concentration analysis. Anticoagulated (EDTA) samples were refrigerated and used to perform CBCs by use of an automated analyzer[b] within 24 hours after collection. In part 1, WBC differential counts were manually determined from a Giemsa-stained thin blood smear. Absolute numbers of WBCs were calculated (eg, percentage of neutrophils × total WBC count), and N:L was determined (absolute neutrophil count/absolute lymphocyte count). In part 2, WBC differential and absolute counts were performed with the automated analyzer. Plasma cortisol concentration was measured in duplicate with a standard radioimmunoassay[c] as described elsewhere.[29,32] Intraassay and interassay coefficients of variation were 7.3% and 19.5%, respectively.

Fecal sample collection and analysis—Samples of naturally voided feces were collected from dogs, placed on ice, and transported back to the laboratory for analysis. The centrifugal sucrose flotation procedure[34] was used to identify parasite ova in fecal samples as roundworm, hookworm, whipworm, tapeworm, or coccidian. To provide an estimate of the degree of parasite shed-

ding, each fecal sample was scored from 0 (no ova visible) to 4 (highest numbers of ova visible; **Appendix**).

Statistical analysis—Parametric tests were the preferred means of analysis of study data. When variance was not homogenous as determined by the Levene test, data were transformed logarithmically; when logarithmic transformation was ineffective, square root transformation was performed. For ease of presentation, raw data were used to create all figures. Nonparametric tests were used for data that did not meet assumptions for parametric analysis. All analyses were performed with statistical software,[d] and values of $P < 0.05$ were considered significant.

For data from part 1 of the study, a 2 × 3 (sex by day) ANOVA with day treated as a repeated measure was used to assess differences in WBC counts and plasma cortisol concentration of male and female dogs with increasing shelter duration. Huynh-Feldt correction was applied when sphericity was significant ($P < 0.01$). Values for shelter dogs on day 1 were compared with those for control dogs (which had been evaluated only once) with the Student *t* test or Mann-Whitney *U* test, as appropriate. When values changed with time for shelter dogs, additional comparisons between shelter dogs on later days and control dogs were sometimes made. Initial review of fecal results indicated similar patterns for male and female dogs, so these data were combined. The McNemar test was used to assess changes in the prevalence of intestinal parasite shedding over time, and the Fisher test was used to compare prevalence between shelter dogs and control dogs. Degree of fecal shedding of parasites was evaluated for changes over time by means of Friedman 2-way ANOVA, and the Mann-Whitney *U* test was used to compare these data with those of control dogs.

Because no significant differences were identified between the sexes for any variable in part 1, data from male and female dogs in part 2 were combined. For data from part 2, a 3 × 2 (treatment group by day) ANOVA was used, with the variable day treated as a repeated measure to assess differences in WBC counts among treatment groups and over time, whereas cortisol concentrations were assessed with a 3 × 2 × 2 (treatment group by day by assessment point [before vs after treatment session]) ANOVA, with day and assessment point treated as repeated measures. Significant interactions were further evaluated with tests for simple main effects.[35] Post hoc paired comparisons were assessed by means of the Newman-Keuls test. For fecal variables, changes over time were evaluated with the McNemar test for shedding prevalence and with Wilcoxon paired comparisons for degree of shedding (shedding scores). Comparisons among treatment groups on each day were made with the χ^2 test for shedding prevalence and with the Kruskal-Wallis test for degree of shedding.

Results

Animals—During the period that dogs were considered for inclusion in the study (3 d/wk for a total of 5 months), approximately 1,000 dogs entered the shelter. Of those 1,000, 92 (9%) dogs (50 males and 42 females) met the inclusion criteria and were enrolled. Body weights ranged from 5.2 to 37.8 kg (11.4 to 83.2 lb). Estimated ages ranged between 6 months and 7 years. Fifty-four (59%) dogs appeared to be purebred. The most common of those breeds included pit bull–type dog, German Shepherd Dog, and Labrador Retriever, with 60 (65%) dogs classified as 1 of these 3 breeds.

Of the 92 shelter dogs, 40 were enrolled in part 1 of the study to evaluate plasma cortisol concentration, WBC values, and fecal shedding of parasites at various points during shelter housing. Seven of those dogs were claimed by an owner prior to study completion, leaving a final group size of 33 shelter dogs that received no experimental manipulation other than venipuncture. Fifteen privately owned dogs (9 females and 6 males) qualified for inclusion in the control group for part 1. Ages ranged from 6 months to 7 years, and body weight ranged from 9.5 to 54.5 kg (21.0 to 120.0 lb). Approximately half of control dogs were purebred, and half were of mixed breed, with Labrador Retriever, Beagle, and pit bull–type dog most commonly represented.

The remaining 52 shelter dogs were enrolled in part 2 of the study to evaluate the effect of daily 30-minute petting sessions on variables assessed in part 1. Four of those dogs were claimed by their owners prior to study completion, and 1 dog did not complete the study because it developed gastrointestinal disease requiring medical intervention, leaving a final group size of 47.

Some shelter dogs in both parts of the study developed clinical signs of upper respiratory tract infection during the 10-day housing periods. During part 1 of the study, 6 of 40 (15%) dogs were affected, and during part 2, 10 of 52 (19%) dogs were affected. All infections were clinically mild and not associated with systemic signs, and no dog met the requirements for intervention per standard shelter policy.

Mean time required for blood sample collection for all dogs in the study was 122 seconds. Most (95%) blood samples were collected in < 4 minutes.

Evaluation of WBC values, plasma cortisol concentration, and fecal shedding of parasites—In part 1 of the study, total leukocyte count ($P = 0.003$), neutrophil count ($P = 0.007$), and lymphocyte count ($P = 0.002$) increased significantly from day 1 (day after arrival at shelter) to day 10 for dogs housed in the animal shelter **(Figure 1)**. However, no significant changes in monocyte count or N:L were identified over time for that group. Compared with control dogs (evaluated once), shelter dogs at day 1 had a significantly higher total leukocyte count ($P < 0.001$), neutrophil count ($P < 0.001$), monocyte count ($P = 0.033$), and N:L ($P < 0.001$). No difference was evident between control dogs and shelter dogs in lymphocyte counts on day 1 or even on day 10, when lymphocyte count reached its highest value for shelter dogs.

Plasma cortisol concentration was highest in shelter dogs on day 1 and decreased significantly ($P = 0.008$) over the 10-day monitoring period **(Figure 2)**. Cortisol concentrations were also significantly ($P < 0.001$) higher for shelter dogs on day 1 than for control dogs. Even on day 10, when cortisol concentrations were lowest in shelter dogs, values still were significantly ($P = 0.001$) higher than values for control dogs.

On any given measurement day during the 10-day monitoring period, between 35% and 50% of shelter

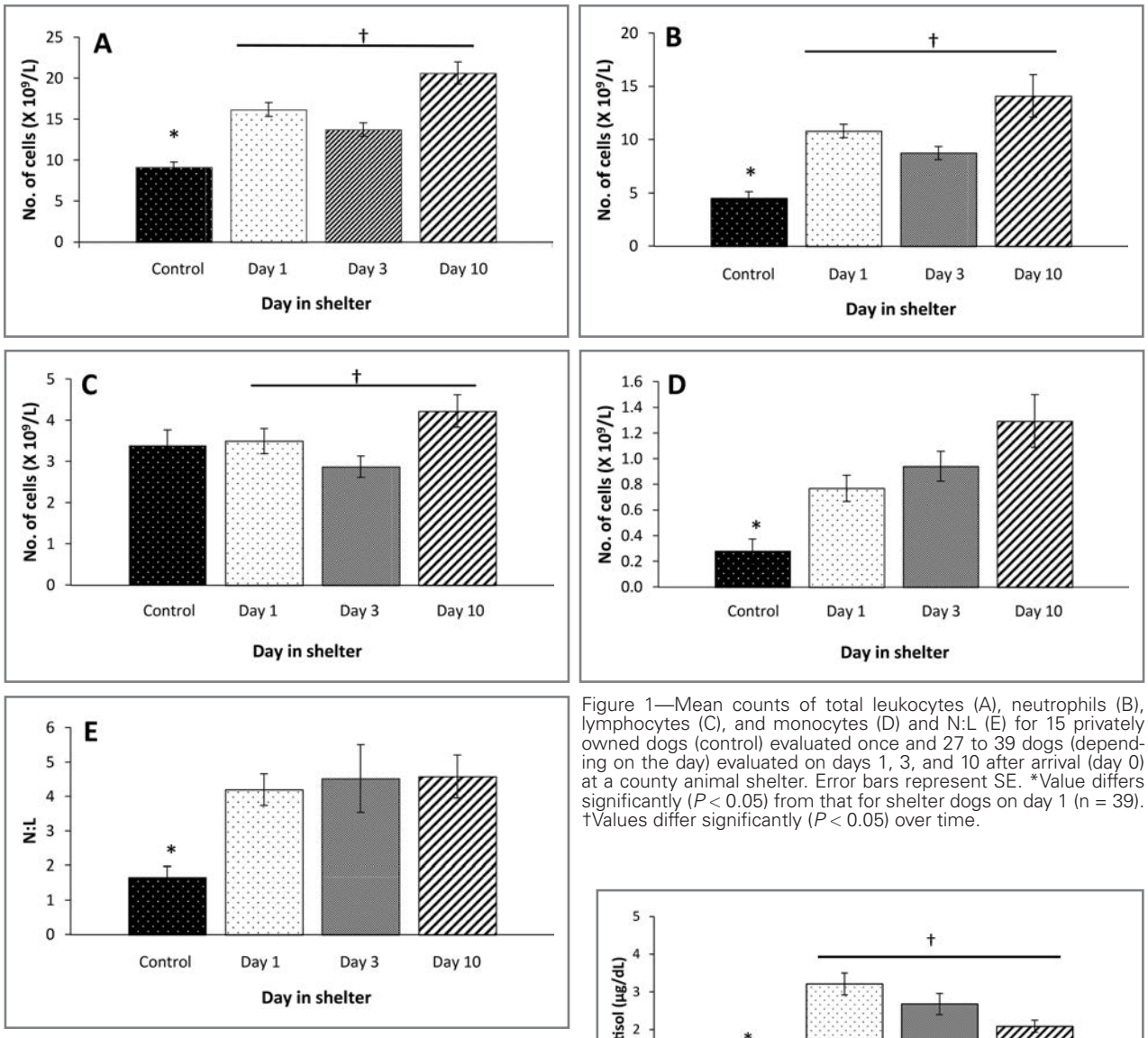

Figure 1—Mean counts of total leukocytes (A), neutrophils (B), lymphocytes (C), and monocytes (D) and N:L (E) for 15 privately owned dogs (control) evaluated once and 27 to 39 dogs (depending on the day) evaluated on days 1, 3, and 10 after arrival (day 0) at a county animal shelter. Error bars represent SE. *Value differs significantly ($P < 0.05$) from that for shelter dogs on day 1 (n = 39). †Values differ significantly ($P < 0.05$) over time.

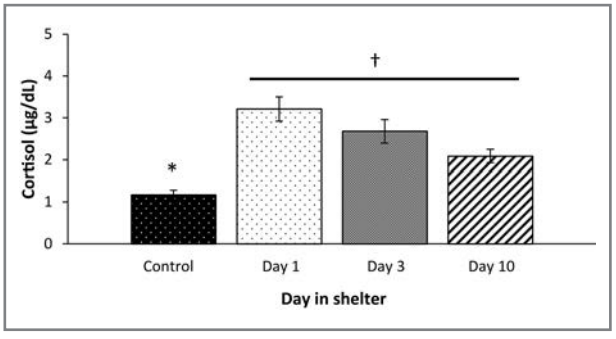

Figure 2—Mean plasma cortisol concentrations of the dogs in Figure 1 (n = 28). *Value differs significantly ($P < 0.05$) from all 3 values for shelter dogs. †Values differ significantly ($P < 0.05$) with time.

dogs were identified as shedding intestinal parasite ova in their feces (**Figure 3**). Of dogs shedding parasites, 30% (6/20) were infected with multiple parasite species; a single parasite species was identified for the remainder (14/20). Parasites identified (from most to least common) included whipworms, hookworms, roundworms, and tapeworms. Number of days in the shelter did not have a significant effect on parasite prevalence nor did it affect degree of parasite shedding. Of dogs with parasite ova in their feces, 17% were considered heavy shedders (ie, given a shedding score of 4/4) on day 1, 33% on day 3, and 25% on day 10. Fecal parasite shedding was significantly higher for shelter dogs than for control dogs, not only on day 1 ($P = 0.017$) but also on the day on which the fewest shelter dogs were identified as shedding parasites (day 3; $P = 0.043$).

Evaluation of the effects of petting sessions on study variables—In part 2 of the study, a significant main effect of day on WBC values was identified, with the highest values achieved on day 10 for total leukocyte count ($P < 0.001$), neutrophil count ($P < 0.001$), lymphocyte count ($P = 0.023$), monocyte count ($P < 0.001$), and N:L ($P < 0.001$). A significant ($P = 0.018$) main effect of treatment group was identified for neutrophil count, which was higher for shelter dogs returned to their home kennel for 30 minutes than for shelter dogs kept in a novel room or petted for 30 minutes, and this association was maintained regardless of whether it was the first or tenth day in the shelter. There was no evidence that daily petting sessions affected any WBC value (**Figure 4**).

The ANOVA for plasma cortisol concentration yielded significant main effects for day ($P < 0.001$) and

assessment point ($P = 0.035$; **Figure 5**). These main effects were qualified by significant interactions of treatment group by day ($P = 0.015$), treatment group by assessment point ($P < 0.001$), and day by assessment point ($P = 0.034$). Further analysis of the treatment group-by-day interaction with simple main effects identified no significant effect of treatment group on day 1 or day 10. For the interaction of day by assessment point, simple main effects revealed that across treatment groups, there was a significant ($P = 0.001$) decrease in cortisol concentration from before the treatment session to after the treatment session on day 10 (before, 1.76 ± 0.12 µg/dL; after, 1.48 ± 0.17 µg/dL) but not on day 1 (before, 2.86 ± 0.38 µg/dL; after, 3.09 ± 0.37 µg/dL). Finally, for the interaction of treatment group by assessment point, simple main effects revealed that petting resulted in a significant ($P = 0.003$) decrease in plasma cortisol concentration from before the treatment session to after the treatment session regardless of day of measurement, whereas no such significant change was identified for dogs in the home-kennel or novel-room groups.

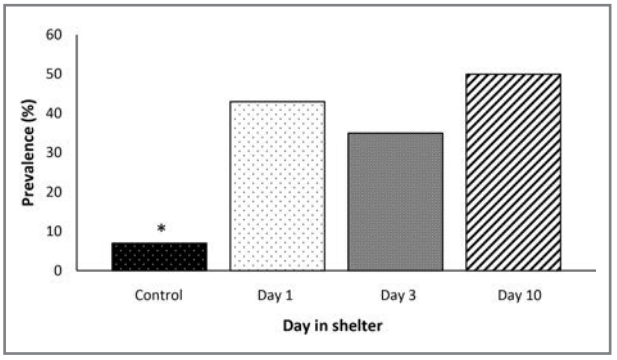

Figure 3—Prevalence of fecal shedding of ova from 1 or more parasite species for the dogs in Figure 1. *Value is significantly lower than values for shelter dogs at any assessment point. *See* Figure 1 for remainder of key.

Similar to the results in part 1 of the study, infection with multiple intestinal parasites was common in the shelter dogs. Parasites detected (from most common to least common) included whipworms, hook-

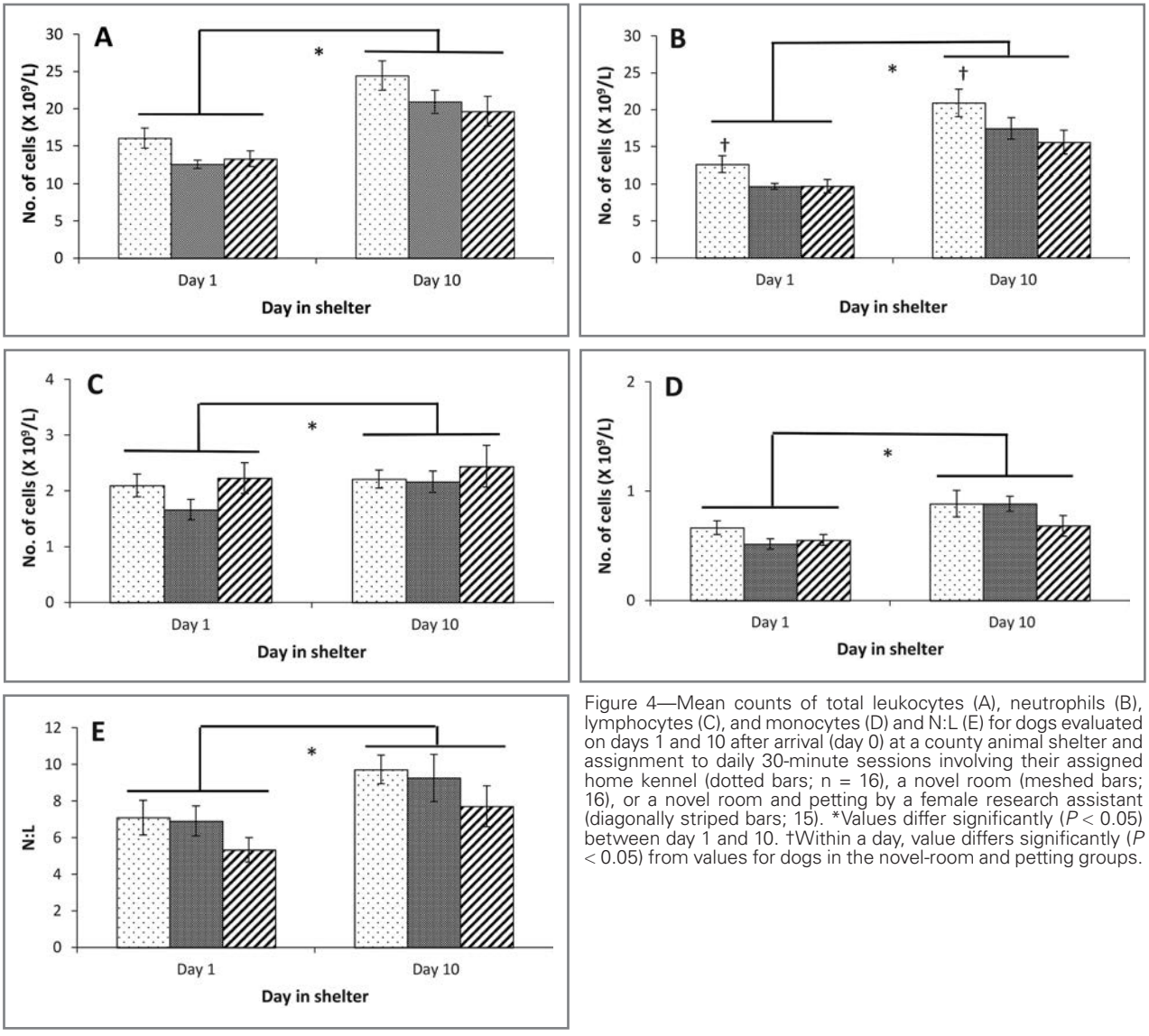

Figure 4—Mean counts of total leukocytes (A), neutrophils (B), lymphocytes (C), and monocytes (D) and N:L (E) for dogs evaluated on days 1 and 10 after arrival (day 0) at a county animal shelter and assignment to daily 30-minute sessions involving their assigned home kennel (dotted bars; n = 16), a novel room (meshed bars; 16), or a novel room and petting by a female research assistant (diagonally striped bars; 15). *Values differ significantly ($P < 0.05$) between day 1 and 10. †Within a day, value differs significantly ($P < 0.05$) from values for dogs in the novel-room and petting groups.

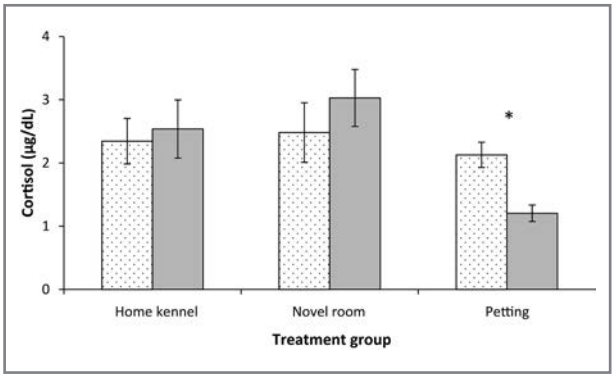

Figure 5—Mean plasma cortisol concentrations of the dogs in Figure 4 as measured before (dotted bars) and after (gray bars) the 3 types of 30-minute treatment sessions. Mean values include measurements obtained on days 1 and 10 after shelter arrival. *Values for dogs in the petting group differ significantly ($P < 0.05$) between before and after the petting session.

worms, roundworms, and coccidia. No differences were identified in percentages of dogs shedding parasite ova in their feces among treatment groups on day 1 or 10, nor did percentages differ between day 1 and 10 for any treatment group. Overall, results of the first fecal examination (performed on day 1, 2, or 3) indicated that 43% (21/49) of all dogs were shedding parasite ova in their feces, whereas on day 10, 37% (15/41) were shedding parasites. No difference in the degree of intestinal parasite shedding was identified among treatment groups on day 1 or 10. However, degree of shedding decreased significantly ($P = 0.025$) between days 1 and 10 for dogs in the novel-room group (but not for dogs in the other 2 groups). Of all dogs shedding parasites on day 1 after shelter arrival, 29% (6/21; 5 dogs in the petting group and 1 dog in the home-kennel group) were considered heavy shedders. On day 10, only 20% (3/15) of dogs shedding parasites were considered heavy shedders, including 1 dog in the petting group and 2 dogs in the home-kennel group. Nonetheless, there was no statistical evidence that daily petting sessions reduced intestinal parasite shedding in the shelter dogs.

Discussion

High WBC counts were identified in dogs housed in an animal shelter in the present study, and certain leukocyte counts increased during the 10-day period after shelter admission. Compared with privately owned dogs assessed once, shelter dogs had significantly higher total leukocyte, neutrophil, and monocyte counts the first day after shelter admission (day 1). In both the observational portion (comparison between shelter dogs and control dogs and within shelter dogs over time) and experimental portion (comparison among treatment groups of shelter dogs and within treatment groups over time) of the study, total leukocyte, neutrophil, and lymphocyte counts for shelter dogs were significantly greater on day 10 than on day 1; in the experimental portion, monocyte count also increased significantly with time. Although the stress associated with shelter housing has been studied in dogs for many years, the effect of shelter housing on immune activity has not, to the authors' knowledge, been experimentally evaluated. The study reported here yielded the first evidence that WBC counts increased as early as approximately 24 hours after arrival to an animal shelter and continued to increase with time. These increases were likely attributable to a physiological stress response of the dogs, but infectious processes (had they been detected) could have also contributed to some of the changes detected.

Many shelter dogs in the present study had parasitic infections. We were unable to determine the extent to which regular administration of parasiticides to the privately owned control dogs might have contributed to differences observed in parasite shedding values between those dogs and the shelter dogs. At any given assessment point during 10 days of shelter housing, 35% to 50% of shelter dogs were identified as shedding parasite ova in their feces, and infection with multiple parasites was common. These findings were consistent with those of a previous report[3] of parasite prevalence in shelter dogs in the Midwestern region of the United States. The nature of the upper respiratory tract infections identified in some shelter dogs in both parts of the study was not identified and might have been viral, bacterial, or both. Bacterial infections may cause increases in numbers of circulating neutrophils, and indeed, neutrophil count increased significantly over time in the shelter dogs. For the statistical analyses in both portions of the study, data from all dogs were included because we believed this would accurately represent a typical shelter population. However, analysis was also performed without data from dogs that developed signs of illness over the course of the study, and significant differences reported here remained. It is questionable whether parasitic infections or respiratory infections affected the WBC counts in the present study.

On arrival to the shelter, dogs were vaccinated in a manner consistent with recommendations for shelter dog management.[36] In a previous study[37] involving healthy 1- to 5-year-old dogs, the immune response during the 10-day period after vaccination with inactivated canine parvovirus, modified-live canine distemper virus, and modified-live canine adenovirus 1 vaccine led to a decrease in numbers of lymphocytes and total leukocytes, but in a different study[38] involving vaccination of 3- to 7-month-old dogs with modified-live canine parvovirus vaccine, no effects on leukocyte numbers were identified. Therefore, it does not appear that vaccination could account for the increase in leukocytes observed over time in the present study.

As expected, plasma cortisol concentration of the shelter dogs in the present study was higher than that of control dogs, and this concentration decreased over the 10-day monitoring period in both portions of the study. These findings were consistent with results of a previous study[25] performed in the authors' laboratory. Cortisol induces multiple changes in numbers of circulating WBCs through redistribution between blood and tissues.[12] Changes primar-

ily include an approximate doubling of numbers of circulating neutrophils and a decrease in circulating lymphocytes, although increases in circulating monocytes are less consistently identified in dogs.[11,12] Total leukocyte count increases primarily as a result of the increase in neutrophils, and calculation of the N:L provides an objective measurement that can be used to assess physiologic stress.[39] In clinically normal dogs, this ratio is approximately 3:1.[40] The N:L of shelter dogs ranged from 4:1 to 5:1 on any given day evaluated in the present study, which was more than double the N:L of control dogs. Interestingly, although plasma cortisol concentration decreased in shelter dogs over the 10-day monitoring period, N:L did not. The N:L is reportedly a more accurate indicator of chronic stress than corticosterone concentration in rats[41] and may also indicate a continued high degree of stress in shelter dogs.

Compared with control dogs, shelter dogs had higher total leukocyte, neutrophil, and monocyte counts on day 1. This finding is consistent with those of previous studies[7,42] conducted to evaluate the peripheral leukocyte response to stressors in dogs. By day 10 in the present study, shelter dogs had further increases in total leukocyte, neutrophil, and monocyte counts (although monocyte count only increased in experiment portion of the study). However, the change observed in lymphocyte count was unexpected. Although cortisol causes a decrease in the number of circulating lymphocytes,[12] dogs in the present study had an increase in lymphocyte count while in the shelter. Lymphocyte count did not differ significantly between shelter dogs on day 1 and control dogs, although plasma cortisol concentrations of shelter dogs at the same point were significantly higher than in control dogs. We suspected that the catecholamine response of the SAM axis affected leukocyte distribution as well. Catecholamine release leads to increases in neutrophil, monocyte, and lymphocyte counts. It is likely that the stress associated with shelter housing stimulated both the SAM and HPA axes and that the WBC response was attributable to the combination of stress hormones.

As little as 30 minutes of daily petting in a quiet room, but not exposure to the quiet room alone, resulted in a significant decrease in plasma cortisol concentration in shelter dogs. This effect was consistent with previous findings that cortisol concentration decreases after a sole petting session given shortly after dog arrival at a shelter.[29] Decreases in plasma cortisol concentration from before to after petting on days 1 and 10 in the present study indicated that the benefits of petting continued beyond the period after arrival to a shelter, when cortisol concentrations were highest.

We hypothesized that daily petting sessions would reduce the stress response of shelter dogs and therefore reduce the elevated total leukocyte, neutrophil, and monocyte counts and N:L observed in part 1 of the study. As expected, dogs in the home-kennel group (which did not receive daily petting) had significantly higher neutrophil counts on day 10 than did dogs in the other treatment groups; however, dogs in the home-kennel group also had higher neutrophil counts on day 1, suggesting that this apparent effect of experimental manipulation was likely spurious. Dogs in the petting group did not have any significant differences in leukocyte count, compared with counts for dogs in the other treatment groups. The lack of effect of petting suggested that other factors in addition to or instead of cortisol influenced the observed changes in immune activity.

The percentage of shelter dogs shedding ova from 1 or more parasite species and the degree of that shedding were evaluated over the 10-day monitoring period, and no significant difference among treatment groups was identified on any day. However, when each treatment group was evaluated separately to compare the degree of parasite shedding between days 1 and 10, a significant difference was identified for dogs in the novel-room group, which shed fewer parasites on day 10 than on day 1. We suspected that this decrease in parasite shedding was incidental; however, it may have reflected an unknown effect of the novel room. Evaluation of multiple fecal samples collected on different days from each dog revealed that 10% to 20% of shelter dogs were only intermittently shedding parasite ova. That finding supported recommendations to universally provide parasite treatment to dogs on arrival to a shelter,[36] rather than to provide treatment solely on the basis of the results of 1 fecal examination. Many dogs in the present study were shedding parasite ova in numbers too numerous to count, which could have quickly led to excessive environmental contamination and potential transmission to uninfected shelter dogs. The prepatent periods of intestinal parasites commonly identified were all > 10 days,[43] precluding the possibility of shelter-acquired infection in dogs in the present study. Shedding of parasite ova while in the shelter was likely affected by immune system function, and we suspected that this would be impacted by the stressors associated with shelter housing. Canine hookworm and roundworm infections are zoonoses capable of causing sometimes substantial disease in humans,[20] further supporting the need for effective hygiene and sanitation protocols while caring for shelter dogs.

Several limitations may have affected outcomes in the study reported here. The animal shelter environment posed several challenges, and much consideration was given to controlling extraneous variables. To minimize variation in the treatment of dogs by shelter staff, an overview of the research procedures and goals was provided to the staff before the study began. Research activities were scheduled around cleaning activities, but at times these coincided. The dogs evaluated were of unknown background, and despite their outwardly healthy appearance, undetected underlying health disorders might have existed. Sample sizes were chosen on the basis of available parasite prevalence and plasma cortisol data[3,29]; however, sample sizes may have been inadequate to detect differences in WBC counts. Initial observation of the range of parasite shedding among dogs in the shelter prompted us to include a variable to estimate the degree (rather than just prevalence) of shedding, but that variable must be considered only a

rough estimate. Study duration was another limitation. Although 10 days was the limit to practically evaluate the dogs given the constraints of the shelter operation, this period was too brief to allow full evaluation of infection and immune activity variables. Furthermore, because animal shelter design and management techniques can vary among facilities, the applicability of our findings to other shelters is unknown.

Regardless of the aforementioned limitations, the study reported here provided insight into the extent of physiologic changes in dogs housed in animal shelters. Plasma cortisol concentration as well as behavioral variables reflect elements of the welfare of shelter dogs,[44,45] so implementation of procedures known to reduce cortisol responses and normalize behavior is desirable for shelter environments. Nevertheless, daily 30-minute petting sessions in the present study, although effective for reducing increases in cortisol concentration, had no measureable effect on WBC count or parasite shedding of shelter dogs. Additional research is needed to identify steps that could be taken to more fully mitigate the consequences of shelter housing on dogs.

a. Duramune Max5 and Bronchi-Shield III, Boehringer Ingelheim, Ridgefield, Conn.
b. HMII and HM5, Abaxis Inc, Union City, Calif.
c. Cortisol Coat-a-Count, Siemens, Los Angeles, Calif.
d. SPSS, version 21, IBM Corp, Armonk, NY.

References

1. American Society for the Prevention of Cruelty to Animals. Pet statistics. Available at: www.aspca.org/about-us/faq/pet-statistics. Accessed Sep 5, 2014.
2. Asilomar Accords. Participating organizations. Available at: www.asilomaraccords.org/participating_organizations.html. Accessed Oct 24, 2013.
3. Blagburn BL, Lindsay DS, Vaughan JL, et al. Prevalence of canine parasites based on fecal flotation. *Compend Contin Educ Pract Vet* 1996;18:483–509.
4. Lord LK, Reider L, Herron ME, et al. Health and behavior problems in dogs and cats one week and one month after adoption from animal shelters. *J Am Vet Med Assoc* 2008;233:1715–1722.
5. Gingrich E, Lappin M. Practical overview of common infectious disease agents. In: Miller L, Zawistowski S, eds. *Shelter medicine for veterinarians and staff*. 2nd ed. Ames, Iowa: Wiley-Blackwell, 2013;297–328.
6. Goldstein DS. *The autonomic nervous system in health and disease*. New York: Marcel Dekker Inc, 2001.
7. Beerda B, Schilder MB, van Hooff JA, et al. Manifestations of chronic and acute stress in dogs. *Appl Anim Behav Sci* 1997;52:307–319.
8. Padgett DA, Glaser R. How stress influences the immune response. *Trends Immunol* 2003;24:444–448.
9. Dhabhar FS. Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. *Allergy Asthma Clin Immunol* 2009;16:300–317.
10. Dhabhar FS, Malarkey WB, Neri E, et al. Stress-induced redistribution of immune cells—from barracks to boulevards to battlefields: a tale of three hormones—Curt Richter Award winner. *Psychoneuroendocrinology* 2012;37:1345–1368.
11. Thrall MA. *Veterinary hematology and clinical chemistry*. Philadelphia: Lippincott Williams & Wilkins, 2004.
12. Dhabhar FS, Miller AH, McEwen BS, et al. Effects of stress on immune cell distribution—dynamics and hormonal mechanisms. *J Immunol* 1995;154:5511–5527.
13. Glaser R, Rice J, Speicher CE, et al. Stress depresses interferon production by leukocytes concomitant with a decrease in natural killer cell activity. *Behav Neurosci* 1986;100:675–678.
14. Ghoneum M, Gill G, Assanah P, et al. Susceptibility of natural killer cell activity of old rats to stress. *Immunology* 1987;60:461–465.
15. Cohen S. Keynote presentation at the eighth international congress of behavioral medicine. *Int J Behav Med* 2005;12:123–131.
16. Rozlog LA, Kiecolt-Glaser JK, Marucha PT, et al. Stress and immunity: implications for viral disease and wound healing. *J Periodontol* 1999;70:786–792.
17. Webster Marketon JI, Glaser R. Stress hormones and immune function. *Cell Immunol* 2008;252:16–26.
18. Rojas IG, Padgett DA, Sheridan JF, et al. Stress-induced susceptibility to bacterial infection during cutaneous wound healing. *Brain Behav Immun* 2002;16:74–84.
19. Hunzeker J, Padgett DA, Sheridan PA, et al. Modulation of natural killer cell activity by restraint stress during an influenza A/PR8 infection in mice. *Brain Behav Immun* 2004;18:526–535.
20. Markell EK, Voge M, John DT. *Medical parasitology*. Philadelphia: WB Saunders Co, 1992.
21. Voith VL, Borchelt PL. Separation anxiety in dogs. *Compend Contin Educ Pract Vet* 1985;42:42–52.
22. Menor-Campos DJ, Molleda-Carbonell JM, López-Rodriguez R. Effects of exercise and human contact on animal welfare in a dog shelter. *Vet Rec* 2011;169:388.
23. Bergamasco L, Osella MC, Savarino P, et al. Heart rate variability and saliva cortisol assessment in shelter dog: human-animal interaction effects. *Appl Anim Behav Sci* 2010;125:56–68.
24. Herron ME, Kirby-Madden TM, Lord LK. Effects of environmental enrichment on the behavior of shelter dogs. *J Am Vet Med Assoc* 2014;244:687–692.
25. Hennessy MB, Davis HN, Williams MT, et al. Plasma cortisol levels of dogs at a county animal shelter. *Physiol Behav* 1997;62:485–490.
26. Tanaka A, Wagner DC, Kass PH, et al. Associations among weight loss, stress, and upper respiratory tract infection in shelter cats. *J Am Vet Med Assoc* 2012;240:570–576.
27. Coppola CL, Grandin T, Enns RM. Human interaction and cortisol: can human contact reduce stress for shelter dogs? *Physiol Behav* 2006;87:537–541.
28. Hennessy MB, Williams MT, Miller DD, et al. Influence of male and female petters on plasma cortisol and behaviour: can human interaction reduce the stress of dogs in a public animal shelter? *Appl Anim Behav Sci* 1998;61:63–77.
29. Shiverdecker MD, Schiml PA, Hennessy MB. Human interaction moderates plasma cortisol and behavioral responses of dogs to shelter housing. *Physiol Behav* 2013;109:75–79.
30. West-Hyde L, Floyd M. Dentistry. In: Ettinger SJ, Feldman EC, eds. *Textbook of veterinary internal medicine*. 4th ed. Philadelphia: WB Saunders Co, 1995;1097–1124.
31. Ofri R. Lens. In: Maggs JF, Miller PE, Ofri R, eds. *Slatter's fundamentals of veterinary ophthalmology*. 5th ed. St Louis: Elsevier Saunders, 2013;272–290.
32. Hennessy MB, Voith VL, Hawke JL, et al. Effects of a program of human interaction and alterations in diet composition on activity of the hypothalamic-pituitary-adrenal axis in dogs housed in a public animal shelter. *J Am Vet Med Assoc* 2002;221:65–71.
33. Tuber DS. Teaching Rover to relax: the soft exercise. *Animal Behavior Consultants Newsletter* 1986;3(1).
34. Blagburn BL, Butler JM. Optimize intestinal parasite detection with centrifugal fecal flotation. *Vet Med* 2006;101:455–464.
35. Winer BJ. *Statistical principles in experimental design*. New York: McGraw-Hill Book Co, 1971.
36. Newbury S, Blinn MK, Bushby PA, et al. Association of Shelter Veterinarians guidelines for standards of care in animal shelters, 2010. Available at: sheltervet.org/wp-content/uploads/2012/08/Shelter-Standards-Oct2011-wForward.pdf. Accessed Apr 18, 2014.
37. Miyamoto T, Taura Y, Une S, et al. Changes in blastogenic responses of lymphocytes and delayed type hypersensitivity responses after vaccination in dogs. *J Vet Med Sci* 1992;54:945–950.
38. Phillips TR, Schultz RD. Failure of vaccine or virulent strains of canine parvovirus to induce immunosuppressive effects on the immune system of the dog. *Viral Immunol* 1987;1:135–144.
39. Davis AK, Maney DL, Maerz JC. The use of leukocyte profiles to

measure stress in vertebrates: a review for ecologists. *Funct Ecol* 2008;22:760–772.
40. Feldman BF, Thomason KJ. Useful indexes, formulas, and ratios in veterinary laboratory diagnostics. *Compend Contin Educ Pract Vet* 1989;11:169–180.
41. Swan MP, Hickman DL. Evaluation of the neutrophil-lymphocyte ratio as a measure of distress in rats. *Lab Anim (NY)* 2014;43:276–282.
42. Bergeron R, Scott SL, Émond JP, et al. Physiology and behavior of dogs during air transport. *Can J Vet Res* 2002;66:211–216.
43. Companion Animal Parasite Council. CAPC Recommendations. Available at: www.capcvet.org/capc-recommendations/. Accessed Sep 1, 2015.
44. Beerda B, Schilder MB, van Hooff JA, et al. Behavioural, saliva cortisol and heart rate responses to different types of stimuli in dogs. *Appl Anim Behav Sci* 1998;58:365–381.
45. Beerda B, Schilder MB, Van Hooff JA, et al. Behavioural and hormonal indicators of enduring environmental stress in dogs. *Anim Welf* 2000;9:49–62.

Appendix

Classification system used for quantification of degree of fecal shedding of parasites by dogs as evaluated via light microscopy.

0 = Negative, with no ova visible.
1 = Positive, with < 10 ova visible on the slide. Ova could pertain to 1 or more parasite species. Most fields of view on the slide contained no ova.
2 = Positive, with > 10 total ova visible on the slide. Ova could pertain to 1 or more parasite species. Most fields of view contained no ova, but many contained a single ovum and some may have contained groupings of up to 10 to 15 ova.
3 = Positive. Ova could pertain to 1 or more parasite species. Most fields of view contained no ova, but many contained groupings of > 10 to 15 ova.
4 = Positive. Ova could pertain to 1 or more parasite species. Most fields of view contained ova, many with groupings of ova too numerous to count.