Clippers are superior to scissors in the collection of hair for chemical analysis in companion dogs: a Dog Aging Project preliminary study

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OBJECTIVE
To identify the safest, most efficient method for hair sample collection from companion dogs among clippers, scissors, and razors and to validate obtained samples with cortisol concentration analysis.

ANIMALS
25 healthy, privately owned dogs.

METHODOBS
2 hair samples were collected from each dog’s ischiatic region with different implements (scissors, razors, or clippers). The collecting clinician completed a Hair Collection Questionnaire (HCQ) for each sample that compared subjective sample quality, time of collection, restraint needed, and patient experience. Each sample was evaluated by cortisol enzyme immunoassay.

RESULTS
Clippers had higher overall HCQ scores than scissors, and scissors had higher HCQ scores than razors. Collection was faster for clippers than scissors, and scissors were faster than razors. There were no differences in sample quality between scissors and clippers, and sample quality was lower with razors. There was no difference in restraint needed or patient experience. Collection of long hair had higher HCQ scores than collection of medium and short hair. Collection of hair from dogs with an undercoat had higher HCQ scores than collection of hair from dogs without an undercoat. Dog size had no effect on HCQ score. Hair cortisol concentration did not vary between scissors or clippers (P = .111). Hair color and age did not affect hair cortisol concentration (P = .966 and P = .676, respectively).

CLINICAL RELEVANCE
Clippers are recommended for hair sample collection from companion dogs. Scissors are an adequate alternative.

Keywords: hair collection, scissors, clippers, dog, cortisol

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cortisol concentrations, thus impacting results.\textsuperscript{9,14–16} By contrast, not only is hair an easily accessible substance that can be collected with minimal restraint, but hair also incorporates compounds over weeks to months and is not influenced by the events of the day of collection, even if restraint were required.\textsuperscript{5,9,10,17–19} Hair sampling provides an alternative perspective when evaluating cortisol, as the values obtained from hair are representative of prolonged periods of time and provide insight regarding chronic stress levels.\textsuperscript{5,9,12,18,19} Finally, hair can be stored for months to years after collection and still provide meaningful results from analysis.\textsuperscript{17}

Despite these valuable attributes of hair analysis, there is no standardized means of collection of hair from dogs, and shaving, cutting, brushing, and convenience collection of shed samples have all been used.\textsuperscript{12,19–21} These techniques can result in a variable amount of hair per sample. Reusable hair collection tools such as electric clippers may be difficult to clean thoroughly before use for hair sample collection and could provide a source of impurities including hair from previous patients or soiling from bodily contaminants, as well as cleaning or oiling solutions. When brushing or plucking is utilized, it could lead to the contaminating presence of hair follicles and associated cellular material or blood within the sample.\textsuperscript{22}

The objective of the study reported here was to identify a simple, reliable method of collecting hair samples from minimally restrained companion dogs, suitable for use by owners or veterinary professionals in a wide variety of settings. Interest in defining this technique was motivated by the Dog Aging Project,\textsuperscript{23} a longitudinal, nationwide study of companion dogs, in which the use of reliable biospecimen collection techniques for participating dogs is needed. Two single-use tools (scissors and disposable razor) and multiuse electric clippers were selected for comparison. Cortisol testing of the samples was then used to confirm whether the collection methods employed provided a sample fit for analytic purposes. We hypothesized that the scissors collection technique would demonstrate superior feasibility of sample collection, including consistent mass of hair collected, hair free from additional contamination due to collection style, and minimal need for subject restraint when compared to the disposable razor or electric clippers.

### Methods

#### Study participants

Healthy, privately owned dogs were recruited from volunteer owners affiliated with the Texas A&M University (TAMU) Veterinary Medical Teaching Hospital. Dogs with a range of subjectively assessed coat lengths (short, medium, and long) were intentionally recruited. Inclusion and exclusion criteria were limited to requirements that the dog be clinically healthy, with no known or clinically suspected concurrent adrenal disease, and be cooperative for brief restraint and hair collection. All procedures were approved by the TAMU IACUC (AUP IACUC 2019-0303 CA). The procedures were determined not to be human subjects research by the TAMU Institutional Review Board (submission IRB2019-0952).

#### Collection of hair

Hair collection was performed at the TAMU Veterinary Medical Teaching Hospital by a single clinician (JBE). Each dog was gently restrained by the owner or another individual. Hair color was recorded. For dogs with more than 1 coat color, the color was recorded as “mixed” with the component colors listed subsequently. For each dog, hair was collected from the right side of the posterior, lateral to the tail in the ischiatic region using 1 instrument, the dog was allowed to relax for 5 minutes, and then an alternate instrument was used to collect hair from the corresponding area on the dog’s left side. A single-blade disposable razor, small rounded-tip grooming scissors, and electric clippers were used. Pairwise collections from each dog to assess the 3 instruments (razor vs scissors, razor vs clippers, and scissors vs clippers) were planned. However, due to obvious poor performance by the razor in the first pairwise comparison against scissors (the razor was often unable to cut through the topcoat and therefore obtained no or poor sample), the razor versus clippers paired collection was not performed. The goal was to collect approximately 200 to 250 mg of hair per site, per dog, which appeared visually as about 2 tablespoons. Hair was stored in small plastic tubes at room temperature until it was sent out for analysis (approx 2 to 3 months).

#### Scoring of hair collection

A questionnaire was created to document the experience of hair collection (Hair Collection Questionnaire [HCQ]) by the clinician obtaining the sample (Supplementary Table S1). For each dog, size class (small, < 10 kg; medium, 10 to 25 kg; and large, > 25 kg) and hair length (short, medium, or long) were recorded. The length of hair was determined by the clinician’s subjective assessment. Additionally, the presence of an undercoat was recorded. Likert-type items were used to rate the experience and success of collection on a scale of 1 to 3; higher scores indicated better performance. The collection process was timed and speed was scored as slow (> 120 seconds), moderate (30 to 120 seconds), or fast (< 30 seconds) (scores of 1, 2, and 3, respectively). Degree of restraint required to complete collection was subjectively scored as substantial (1), moderate (2), or none/minimal (3). The dog’s experience was subjectively scored as substantial discomfort or anxiety (1), mild-moderate discomfort or anxiety (2), or minimal to no discomfort or anxiety (3). Sample quality based on the consistency of hair length and the number of damaged hairs was scored as poor (1) or good (3) and included an option for “no sample obtained” (0). Collection process questions were answered once for each sampling instrument used (ie, twice for each dog) and a cumulative score was generated for each sampling instrument.
Hair analysis

The methods for cortisol assay were based on similar methods used in previous studies.9,10 Hair samples were weighed, washed in isopropanol to remove contaminants and cortisol that may accumulate on the surface of the hair through contact with humans or other animals, dried, and then homogenized by mincing with scissors to eliminate differences in hormone content across the length of the hair segment. A portion of the minced hair was weighed (target per extraction of approx 50 mg) and then pulverized. Methanol (1.5 mL) was added to pulverized hair and tubes were rotated for 18 to 24 hours to extract cortisol from hair into the methanol. Samples were centrifuged, 1 mL of the methanol layer was removed to a fresh tube, and then methanol was allowed to evaporate under a gentle stream of air. The residue containing extracted cortisol was reconstituted in assay buffer. Results were calculated using the hair mass extracted and the assay result and were reported in picograms of cortisol per milligram of hair:

(\text{Assay result in pg/mL} \div \text{weight of hair in mg})
\times (\text{mL methanol added} \div \text{mL methanol dried down})
\times \text{reconstitution buffer volume in mL} = \text{pg/mg}

Cortisol

Two independent extractions were performed for each sample (Figure 1). Each extract was tested on 2 cortisol assay plates, using different dilution factors for the 2 plates to capture imprecision across the assay calibration range. Extracts were tested in duplicate wells on each plate, yielding 4 test results per extract, and eight test results per sample. Cortisol concentration was determined with an in-house cortisol enzyme immunoassay. This allowed testing of the nested sources of variability, with estimated imprecision calculated from the cortisol results as coefficient of variation (CV) across collection methods, replicate extractions, and between and within assay batches. A maximum CV of 10% was considered an acceptable variation.

Statistical analysis

All statistical analyses were performed in the program R (version 4.2.1; www.r-project.org; The R Foundation), and due to multiple comparisons across the study, we used a Bonferroni correction to set a conservative \( P \) value of .0031 to determine significant differences. We were first interested in determining how scores on the HCQ compared across scissors, clippers, and razors. We used a paired Wilcoxon signed rank test comparing scissors with either clippers or razors for each individual factor (time, restraint, patient experience, and sample quality), as well as overall HCQ scores. We then looked specifically at scissors (due to the larger sample size) to determine if hair type, color, dog size, or presence of an undercoat was associated with HCQ score using a 1-way ANOVA with a Tukey honest significant difference post hoc test if appropriate. Next, we compared cortisol values measured

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**Figure 1**—Hair sample usage and allocation for cortisol analysis and the average coefficient of variation (CV) from 25 companion dogs. Each hair sample \( n = 37 \) was subjected to cortisol extract to yield 2 extracts. Two hair samples only provided sufficient material for 1 extraction \( n = 37 \) for extract 1 and 35 for extract 2). Each extract was split into 2 plates and tested with different dilution factors. Each plate was split into 2 wells for duplicate testing, therefore producing eight cortisol concentration results per sample. The average CV from each sample is listed across all levels of analysis (extracts, plates, and wells) for scissors, clippers, and scissors and clippers combined into the overall CV.
by clippers and scissors using a paired t test. Finally, we evaluated the association of cortisol values with hair color(s) and age using a linear model.

**Results**

**Study participants**

A total of 25 dogs were sampled in this study. There were no significant comorbidities in any of the dogs, aside from 1 dog who had historically diagnosed and reportedly well-regulated hypothyroidism. The most common breed was the pit bull (n = 6), and numerous other breeds were sampled in this study, including the Jack Russell Terrier, Pomeranian, Golden Retriever, Labrador Retriever, Australian Shepherd, Great Dane, Dachshund, and multiple mixed breeds. There were 7 “short,” 8 “medium,” and 10 “long” hair. Of these, there were 8 dogs with “short” hair, 12 with “medium” length hair, and 5 with “long” hair. There were 10 dogs with “blonde” fur, 7 “black,” 1 “brown,” 4 with “mixed (blonde/black),” and 1 with “mixed (blonde/brown).” The median age of all sampled dogs was 6 years (range, 1 to 12 years). Thirteen dogs were sampled with the scissors and razor combination, while 12 were sampled with scissors and clippers; thus, only scissors were utilized in every dog (Figure 2).

**HCQ**

All 25 sampled dogs had an HCQ completed to score each tool (Figure 3 and Table 1). Scissors had higher HCQ scores than razors overall. Scissors were found to produce a faster sample collection and better sample quality than razors but were not found to be different from clippers. Clippers and razors could not be directly compared due to a lack of paired sampling. There were no significant differences in restraint needed for either pairwise sample collection. We could not determine differences in patient experience because the values were all too similar, thus suggesting they were the same.

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**Figure 2**—Hair samples obtained from 25 companion dogs. Participants, pairwise tool comparison, subjective data, and samples collected in the study of hair collection methods from companion dogs.

**Figure 3**—Hair collection questionnaire for 25 companion dogs. Results are displayed for the time needed for hair collection (> 120 seconds = 1, 30 to 120 seconds = 2, or < 30 seconds = 3), restraint required during collection (substantial = 1, moderate = 2, or none/minimal = 3), the overall subjective patient experience (substantial discomfort or anxiety = 1, mild to moderate discomfort or anxiety = 2, or minimal to no discomfort or anxiety = 3) and the quality of sample produced (no sample obtained = 0, poor = 1, or good = 3).
There was no difference in sample quality and between sets of samples collected by each tool extracts, plates, and wells (Figure 1). The CV within than 10% for each point of evaluation amongst the project age and hair cortisol concentrations (P = .676). There was no significant association between hair color and hair cortisol concentration. Due to poor sample quality, no cortisol concentration. Hair of different colors from collection method did not influence the estimates of hair cortisol concentration. Across all dogs and sampling methods, the mean hair cortisol concentration was 9.5 ± 11.1 pg/mg. When specific sampling instruments were considered, there was no significant difference in hair cortisol concentration between samples collected with clippers versus scissors (P = .111 by paired t test). There was no significant association between hair color and cortisol concentration for scissors (P = .995 by 1-way ANOVA) nor was there an association between subject size and hair cortisol concentrations (P = .676). The average CV for all the samples was less than 10% for each point of evaluation amongst the extracts, plates, and wells (Figure 1). The CV within and between sets of samples collected by each tool was also less than 10%.

**Discussion**

Clippers were found to be superior to scissors disproving our hypothesis. Clippers had higher overall HCQ scores than scissors as well as faster collection times. There was no difference in sample quality obtained or dog restraint required between scissors or clipper collection methods. In direct comparison, scissors convincingly outperformed razors in overall HCQ score, time needed for collection, and sample quality produced. Razor performance was so poor (including the inability to obtain adequate sample volume at all) that their use was not continue, precluding direct comparison of cutters versus razors on the same dogs. However, because samples adequate for analysis were consistently obtained by cutters, it is clear that cutters also outperform razors for the purpose of hair collection from companion dogs.

Specific interdog qualities affected the overall sample collection but not because of the implement chosen (razors, scissors, or cutters). Long hair provided higher HCQ scores over short and medium-length hair. These results are unsurprising, as a bigger target (longer hair) makes for easier collection and would be perceived as higher HCQ scores. Additionally, the presence of an undercoat was associated with slightly higher HCQ scores. Dog size did not impact HCQ scores, and there was no association between the tool used and the restraint required to complete collection. Together, these findings confirm that hair collection for analysis is a safe and feasible alternative to other sampling methods for diverse dogs in a clinical setting.

There was no statistical difference in cortisol concentration between hair samples collected by scissors or cutters supporting the fact that both techniques were effective. The CV for cortisol at each point in the analysis for samples collected by scissors versus cutters was similar, suggesting that the collection method did not influence the estimates of cortisol concentration. Hair of different colors from the same and different dogs has previously been shown to have different cortisol concentrations;

**Table 1**—Hair collection questionnaire (HCQ) scores for hair samples collected from 25 companion dogs, 2 samples taken per dog.

<table>
<thead>
<tr>
<th>Tool (HCQ parameter)</th>
<th>Sample size</th>
<th>Mean score</th>
<th>Minimum score</th>
<th>Maximum score</th>
<th>Upper confidence limit</th>
<th>Lower confidence limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scissors (total score)</td>
<td>25</td>
<td>9.6</td>
<td>6</td>
<td>12</td>
<td>10.5</td>
<td>8.8</td>
</tr>
<tr>
<td>Scissors (collection time)</td>
<td>25</td>
<td>2.0</td>
<td>0</td>
<td>3</td>
<td>2.3</td>
<td>1.6</td>
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<tr>
<td>Scissors (sample quality)</td>
<td>25</td>
<td>2.5</td>
<td>1</td>
<td>3</td>
<td>2.8</td>
<td>2.3</td>
</tr>
<tr>
<td>Scissors (patient experience)</td>
<td>25</td>
<td>2.8</td>
<td>2</td>
<td>3</td>
<td>2.9</td>
<td>2.6</td>
</tr>
<tr>
<td>Clippers (total score)</td>
<td>12</td>
<td>11.6</td>
<td>10</td>
<td>12</td>
<td>11.9</td>
<td>11.2</td>
</tr>
<tr>
<td>Clippers (collection time)</td>
<td>12</td>
<td>2.9</td>
<td>2</td>
<td>3</td>
<td>3.1</td>
<td>2.8</td>
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<tr>
<td>Clippers (sample quality)</td>
<td>12</td>
<td>2.8</td>
<td>2</td>
<td>3</td>
<td>3.0</td>
<td>2.6</td>
</tr>
<tr>
<td>Clippers (patient experience)</td>
<td>12</td>
<td>3.0</td>
<td>3</td>
<td>3</td>
<td>3.0</td>
<td>2.6</td>
</tr>
<tr>
<td>Razors (total score)</td>
<td>13</td>
<td>6.6</td>
<td>4</td>
<td>8</td>
<td>7.3</td>
<td>5.9</td>
</tr>
<tr>
<td>Razors (collection time)</td>
<td>13</td>
<td>0.8</td>
<td>0</td>
<td>2</td>
<td>1.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Razors (sample quality)</td>
<td>13</td>
<td>2.5</td>
<td>2</td>
<td>3</td>
<td>2.7</td>
<td>2.2</td>
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<tr>
<td>Razors (patient experience)</td>
<td>13</td>
<td>2.8</td>
<td>2</td>
<td>3</td>
<td>3.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>

The total score is shown for each tool followed by the individual parameters that were graded for each tool. The sample size, mean score, minimum score, maximum score, and upper and lower confidence limits (based on 95% CI) are listed based on the questionnaire that scored the time needed for hair collection (> 120 seconds = 1, 30 to 120 seconds = 2, or < 30 seconds = 3), restraint required during collection (substantial = 1, moderate = 2, or none/minimal = 3), the overall subjective patient experience (substantial discomfort or anxiety = 1, mild to moderate discomfort or anxiety = 2, or minimal to no discomfort or anxiety = 3), and the quality of sample produced (no sample obtained = 0, poor = 1, or good = 3).
however, our study found no difference in cortisol concentrations from hair of different colors or mixed colors from different dogs. Additionally, age was not associated with hair cortisol concentration in the dogs of this study. This agrees with 1 previous study in the Canadian lynx but is counter to another study in feedlot steer that showed older steer had higher hair cortisol concentrations. As previously discussed, hair cortisol concentration is subject to long-term environmental influences that can vary between species and lifestyle. Future studies could evaluate differences in hair cortisol concentration among companion dogs of various ages, hair colors, and lifestyles.

In this study, hair was collected from the same anatomic location (both right and left sides) on each dog, although previous studies have shown differences in hair cortisol concentration from different collection sites. The posterior vertex of the head has been established as the ideal sampling location in human subjects, but no such location has been described for dogs. Some studies have shown that hair cortisol concentrations are similar among different body regions, but other studies involving dogs and other species have documented significant differences in cortisol from hair obtained from various locations. More work is needed to determine whether a standardized location for canine hair sampling for cortisol measurement, or for other analytes of interest, is needed.

The HCQ used ordinal point assignments to identify the domains being assessed as well as an overall assessment. While not a continuous numeric assessment, an ordinal system is appropriate for the experiential observations being collected. The HCQ represented factors that were deemed important by these authors, and it is possible that other clinicians would have chosen other factors to evaluate. Similarly, other clinicians may have weighted their subjective assessments differently based on personal experience or preference. These ordinal scores presented here provide an experiential assessment of the chosen implements and parameters because a more objective or numeric means of assessment is not available.

A limitation of this study was that there was no randomization of the tools, ischiatic region (right or left), or other hair qualities that may have affected HCQ scores (eg, presence of an undercoat, hair length, or size of dog). Additionally, clippers and razors were not used in the same dogs and direct comparisons could not be made. However, despite the initial appeal of razors as inexpensive, single-use instruments, they performed so poorly, including the inability to obtain any sample in many cases, that their use cannot be recommended. The early removal of 1 tool limited the amount of samples and therefore the power obtained by this study. This study was not designed with a sample size selected a priori and was instead an exploratory look at the differences between hair collection tools. Future studies could be designed with an intentional power calculation to determine ideal sample size. Another limitation of this study is that only cooperative dogs were used, which does not reflect the range of dogs in the general population that present to veterinary clinics. While hair collection is nonpainful and does not require excessive restraint, there are still some dogs that may not tolerate hair collection or may require sedation for safety.

The Dog Aging Project is a nationwide longitudinal study of companion dogs. Biological samples for routine and experimental analysis are collected from some participants throughout the country by owners and by veterinarians in various practice settings. To facilitate this process, it was crucial to identify the best method for collection of each of these samples. While sample collection methods for blood and urine are well-defined, canine hair collection is a comparatively novel technique and various methods have been utilized across multiple studies. The goal of this study was to identify an easy-to-use tool that could efficiently provide quality hair samples suitable for analysis from multiple dogs of various hair lengths and body sizes.

We found that clippers provided the best hair samples in the most efficient manner. Scissors also performed well and would be a viable alternative. Both scissors and clippers provided hair samples that were adequate for cortisol analysis.

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Disclosures

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. No AI-assisted technologies were used in the generation of this manuscript.

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Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org