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Short Communication

Transfer and persistence of owner DNA on domestic pets

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ABSTRACT

Domestic pets are frequently present at crime scenes, the homes of victims and suspects, and other persons of interest associated or allegedly associated with a crime. The presence of traces of animal deoxyribonucleic acid (DNA) at a scene can be an investigative lead but could our furry friends also be depositing our DNA along the way? Routine contact with our domestic pets from a pat on the head, kisses, and hugs may all result in transfer of our DNA to an animal. In an effort to explore this possibility, the background human DNA on domestic pets was measured and whether any DNA attributable specifically to the owner of the domestic pet was assessed. Finally, whether the pet would transfer the owner's DNA onto a smooth surface item was also evaluated. Ten domestic pets comprised of felines and canines were utilized for this study. None of the secondary transfer samples produced profiles that would be considered suitable for comparison to known DNA profiles or eligible to be uploaded to a DNA database. Approximately 53 % of all samples collected directly from the pet produced DNA profiles that provided support for the DNA profile under the proposition that the primary pet owner is a contributor rather than an unknown person is to the DNA profile obtained from the sample. However, it is unlikely to have results that support that the source of the DNA is the owner, rather than an unknown person, when the DNA is taken from an item that has been touched by the pet.

1. Introduction

Dogs and cats are, without a doubt, the most owned domestic pets worldwide. In the United States of America alone there is estimated to be approximately 70 million domestic canines and 74 million domestic felines [1]. With this many pets around, it is natural that they are frequently encountered visitors at crime scenes. Traces of pet DNA left at the scene of the crime has proven to be a useful forensic tool and as such, much research exists on this topic [2–11]. The research spans species identification as well as individual pet identification. With increases in the sensitivity of forensic DNA testing the natural question exists: can our pets transfer our DNA to evidence? In order to propose hypotheses addressing these questions an analyst may conduct an analysis and provide an evaluative report, evaluating the DNA evidence under two competing hypotheses. In order to conduct an analysis for the purposes of evaluative reporting, adequate research pertaining to the two hypotheses must be available.

Recently the presence of human DNA on canines and felines has been examined [1–4]. The feline research found that human DNA, typically from the owner of the cat, can be found on the animal; however, this study did not examine transfer of human DNA to other items. Additionally, only cats were examined, and all samples were collected from the right side of the cat. One canine research study involved analyzing both background human DNA recovered from canines as well as the ability of the canines to serve as a vector for transfer of human DNA to a gloved hand, finding that the DNA did indeed transfer from the canine to a gloved hand. The second canine study focused on DNA obtained from canine police dogs after direct transfer of DNA, although the publication acknowledged other probative DNA evidence could be obtained from other modes of transfer from the police dogs [3]. All studies found that human DNA could be recovered from the animals and the canine study demonstrated that canines could also successfully serve as a vector of transfer.

This study consisted of measuring the level of background human DNA present on a domestic pet, whether that DNA was consistent with the primary owner of the domestic pet, and the amount of detectable human DNA transferred from the pet to an item, a plastic card. Canines and felines were chosen due to their predominant selection for domestic pets in the world. The interaction between canines and their owners can vary significantly from the interactions between felines and their

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owners, so it was decided to explore both options. Swabbing was selected over tape-lifting, scraping, or M−Vac® collection to minimize the potential discomfort for the participating animals. Additionally, swabbing enabled collection from the surface of the coat as well as within the coat. Replicate samples were not collected to avoid the potential reduction of DNA available after the initial collection.

2. Methods

2.1. Experimental Design

Ten domestic pets comprising five canines and five felines from various households were utilized. The pets were designated A-J respectively. The felines sampled were three mixed breeds and two savannah cats. The canines were four mid-sized mixed breeds and a Rhodesian Ridgeback. All ten pets are indoor animals, meaning they primarily live indoors with their respective owners. The cats are all solely indoor cats; however, the dogs do go outside for walks routinely. The Rhodesian Ridgeback is the sole pet in the household, all remaining pets live in multi-pet households. All ten pets are allowed on furniture in their respective homes. The cats and dogs are all known to frequently interact with their owners including pats, kisses, and hugs. Dog A lives with two human adults (male/female), Cats B/C and Dog D live with two human adults (male/female), Cats H and J live with one human adult (female) and one human child (female), and Dogs E/F/I and Cat F live with two human adults (females) and two human children (males).

All swabs were collected with fresh single use FitGuard Touch Powder-Free Nitrile gloves for each sample. The swabs were then individually packaged until they were sampled for extraction. All research participants had other humans living in the house; however, many of these individuals were comprised of minor children so the decision was made to focus on the primary owner who could readily provide informed consent. The sampling method utilized for swabbing the pets directly was a single cotton swab rubbed on the area in question. A single swabbing method was utilized to minimize any potential discomfort for the animal. All cotton swabs used for the pet collection were Puritan brand 25–806 1WC Sterile Cotton Tipped Applicators (Guilford, Maine, USA). Three external areas on each pet were separately sampled, the back (from neck to tail), ears/head, and nose/mouth.

For the second part of the experiment, in a separate sampling event, plastic cards were utilized by rubbing the card on a designated area of the animal, again the back, ears/head, and nose/mouth. The cards were rubbed firmly across the entirety of the designated area on both sides of the card. The entirety of the area was sampled, as such the size of the area varied based on the animal. Cards were selected to mimic an animal's brief encounter with a smooth surface item. The plastic cards were Bodno (Lakewood, NJ, USA) Premium brand CR80 30 Mil Graphic Quality PVC cards purchased from Amazon, measuring 8.5725 × 5.3975 cm, and cleaned using a 10 % bleach solution sprayed directly onto the cards prior to use. The cards were then fully allowed to dry for over one hour. The card samples utilized a double swabbing method where the card was first sampled with a wet swab then sampled with a dry swab on each side to ensure any residual moisture was collected from the card. Sample collection for the direct samples occurred in a single event and the card collection was a second event. The number of days between events varied based on availability of the participants from just a week to a few months in time.

Buccal swab collection was used for the primary pet owners. Again, the swabs used were Puritan brand 25–806 1WC Sterile Cotton Tipped Applicators (Guilford, Maine, USA). Swabs were collected by participants vigorously swabbing both sides of the inside of their mouth. The swabs were then individually packaged and stored at room temperature until they were sampled for extraction.

2.2. Sample Processing

All samples were processed by excising the swab tip from the shaft and depositing the cuttings into a single extraction tube for each sample. Samples were then extracted using the Maxwell™ FSC DNA IQ™ Casework Kit with the Promega Casework Extraction Kit processed on a Promega Maxwell® RSC 48 Operating System (Madison, WI, USA). The final elution volume was 50 μL. The Promega PowerQuant® system (Madison, WI, USA) was used for quantification on an Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) using HID Real-Time PCR Analysis Software v2.3.3f2 (Thermo Fisher Scientific, Waltham, MA, USA) and amplified using the Promega PowerPlex® Fusion 6C DNA profiling system (Madison, WI, USA) for 30 cycles on an Applied Biosystems ProFlex™ PCR System thermal cycler (Thermo Fisher Scientific, Waltham, MA, USA). Sample extracts were concentrated to 15 μL using Microcon® DNA Fast Flow Filter (Merck Millipore Ltd., Burlington, MA, USA) for amplification input. Normalization to 1.0 ng was conducted when possible. Quantification and amplification set-up steps were automated on a TECAN Freedom EVO 150 (Zurich, Switzerland). The total nanograms (ng) obtained was calculated by taking the value in ng/μL obtained and multiplying that amount by 50. The total autosomal DNA and the total Y-DNA quantities were assessed. The quantitation values obtained, and whether the values met the routine cut-off, employed by the laboratory the research was conducted at, for the polymerase chain reaction (PCR) amplification for casework, were evaluated. The samples were concentrated after quantitation by Microcon® Filters (Merck Millipore Ltd. Burlington, Massachusetts, USA) when needed (could not normalize to 1 ng without concentration) to 15 μL. Samples with a sufficient quantity of DNA, meeting the internal laboratory threshold cut-off for amplification (0.001 ng/μL) developed during the internal validation for the Promega PowerPlex® Fusion 6C DNA profiling system (Madison, WI, USA), were amplified. It should be noted that utilizing more sensitive DNA profiling systems may impact this cutoff.

DNA profile development was accomplished with capillary electrophoresis on an Applied Biosystems™ 3500xL Genetic Analyzer, data collection with the 3500xL Series Data Collection software v.3 (Thermo Fisher Scientific, Waltham, MA, USA), and analysis using GeneMapper® ID-X software v1.4 (Thermo Fisher Scientific, Waltham, MA, USA). The analytical thresholds used were dye specific at 125 for the blue channel, 119 for the green channel 165 for the yellow channel, 171 for the red channel, and 139 for the purple channel.

If a profile suitable for comparison was obtained it was then compared to the DNA profile of the primary domestic pet owner, who is classified as the individual primarily responsible for the care of the pet. The shedder status of these individuals is unknown. The primary domestic pet owner for all samples was a female. For the purpose of this research female is classified as chromosomal DNA typing results of XX and male is classified as chromosomal DNA typing results of XY [5]. For all pets, additional persons lived in the household and the pets were also exposed to routine visitors such as friends, additional extended family, and package delivery personnel. All pets, except for H and J, had at least one male also living in the home. The primary caretaker was selected in effort to compare to the individual who was primarily responsible for the pet and had the most interaction with the animal. STRmix™ v2.6.02 software (Nichevision Inc., Akron, OH, USA) was used for mixture deconvolutions and likelihood ratio (LR) generation using the following propositions:

Hypothesis 1. (*H*1): *Primary Pet Owner* + *N*-1 *Unknowns*

Hypothesis 2. (*H*2): *N Unknowns*

The suitability of the profile for DNA comparison to known reference standards and, when suitable for comparison, the LR obtained when considering the primary pet owner was analyzed. The minimum standard for suitability for comparison was data at 6 or more non-sex-

determining loci. The number of contributors was determined by evaluating peak height ratios and the minimum number of alleles observed at each locus. A theta value of 3.0 % was selected, the allele frequencies used were from the Expanded Federal Bureau of Investigation (FBI) DNA population database (2016) for the Caucasian, Southeast Hispanic, Southwest Hispanic, and African American/Bahamian/Jamaican populations [6], and the stratified unified LR, was selected.

2.3. Ethics

Human and Animal Research Ethics Clearance for this study was obtained from Griffith University (GU Ref No: 2022/298).

3. Results and Discussion

As the samples in this study were collected from domestic pets, the potential presence of human DNA would demonstrate that the animals had background human DNA, deposited either via direct transfer from a human or indirect transfer, which is to be expected if someone pats their cat or dog, or transfer from the pet's environment. More human DNA was detected on the samples collected from the canines than the felines (Figs. 1-4). This disparity in DNA yield could be attributable to the available surface area of the canines who were inherently larger and/or based on behavior of the pet. The average total human DNA obtained from all feline samples in the 50 µL extract, background and indirect transfer, was 0.06 ng and a standard deviation of 0.11. The average total DNA obtained from all canine samples, background and indirect transfer, was 0.49 ng and a standard deviation of 1.17. This difference equates to over 7 times more DNA on average collected from the canines than the felines. Breaking it down a step further, the average DNA yield from the background samples from felines was 0.11 ng and a standard deviation of 0.14, average indirect transfer from felines was 0.02 ng and a standard deviation of 0.03, average background from canines was 0.98 ng and a standard deviation of 1.52, and average indirect transfer from canines was 0.002 ng and a standard deviation of 0.008. Additionally, a larger variation in the quantity of DNA obtained was observed from the samples. Variation can be attributable to a variety of factors: whether the animal routinely interacts with their owner, whether they are an inside or outside pet, if they sleep in bed with their owner, whether their owner kisses them, and the bathing and cleaning routine of the pet, to name a few. This variation was also observed in previously published research [2]; however, it should be noted that the average quantity of DNA obtained was significantly higher in this study than the 0.060 ng observed in the research by Monkman et al. This may be in part due to the inclusion of additional test sites (sides, chest, and stomach) that had significantly lower recoveries with nearly 30 % of samples resulting in no DNA recovered from the six sites tested. However, it should be noted that a Kruskal-Wallis H test was performed and that distributions were similar for all areas sampled [2].

The total amount of DNA obtained from the samples taken directly from the domestic pet versus the samples of DNA transferred from the domestic pet to the item were compared (Figs. 1-4). In Figs. 1 through 4, the letter before the area is reflective of the designated identifier of the pet sampled. The average combined total quantity of DNA obtained from the samples collected directly from the pets was 0.55 ng. The average combined total quantity of DNA obtained from the samples collected from items that were used to contact the animal was 0.01 ng. This equates to a 40 times higher yield of DNA from the samples collected directly from the domestic pet as opposed to samples collected from the items that came into contact with the domestic pet. Overall, more than 56 % of the secondary transfer samples had no DNA detected. This is comparable to a previous study exclusively with canines where the canine only transferred DNA in 55 % of samples to a plastic sheet [2]. However, evaluating this further only 13 % *(N = 2)* of the canine indirect transfer samples yielded DNA, whereas 73 % *(N = 11)* of the feline indirect transfer samples yielded DNA. These results may be attributable to a higher rate of transfer from the felines. For consideration, the overall yield from the canine background samples was significantly higher than that of the felines. It is possible that differences in the texture of the fur between canines and felines, or the behavior of the animals (such as presence of saliva, dirt, or other foreign material in their fur), results in varying rates of transfer depending on the substrate, i.e. cotton swab versus smooth surface. Similar to how cotton swabs are efficient at picking up DNA but not releasing it back into the tube in contrast to nylon swabs which do not pick up as much DNA but have a higher release of biological material at extraction [7]; however, the successes of both the nylon and cotton swabs are substrate dependent [8]. For comparison, in this experiment the 'substrate' would be the canine or feline. It should be noted, the four samples that did not yield results from the feline indirect transfer samples were from four different cats accounting from three different areas (two back samples, one ears/head, and one nose mouth), as such, there did not appear to be a correlation between the cats themselves or the area sampled.

The total quantity of DNA recovered based on the area of the animal sampled was examined for both cats and dogs (Figs. 1 and 3). Due to the small number of samples obtained from the secondary transfer samples

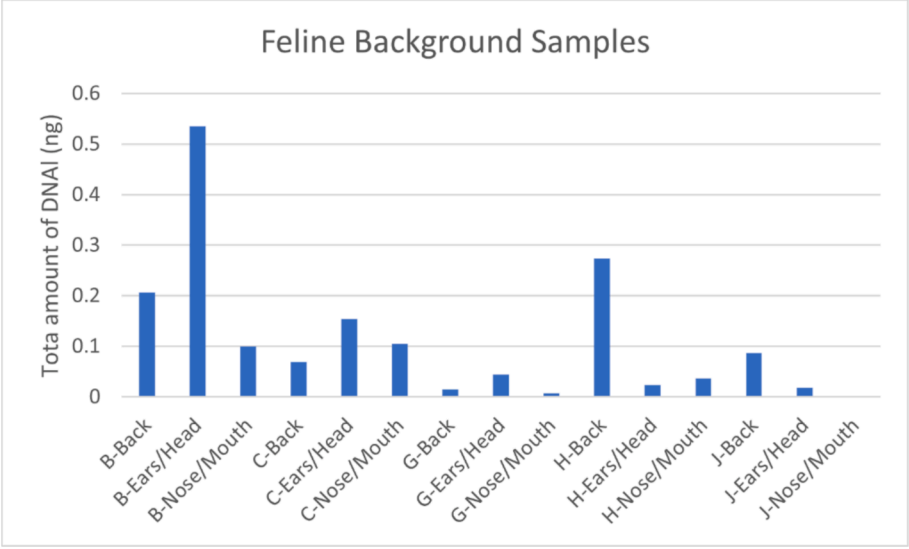

Fig. 1. Total amount of DNA (ng) obtained from the feline background samples.

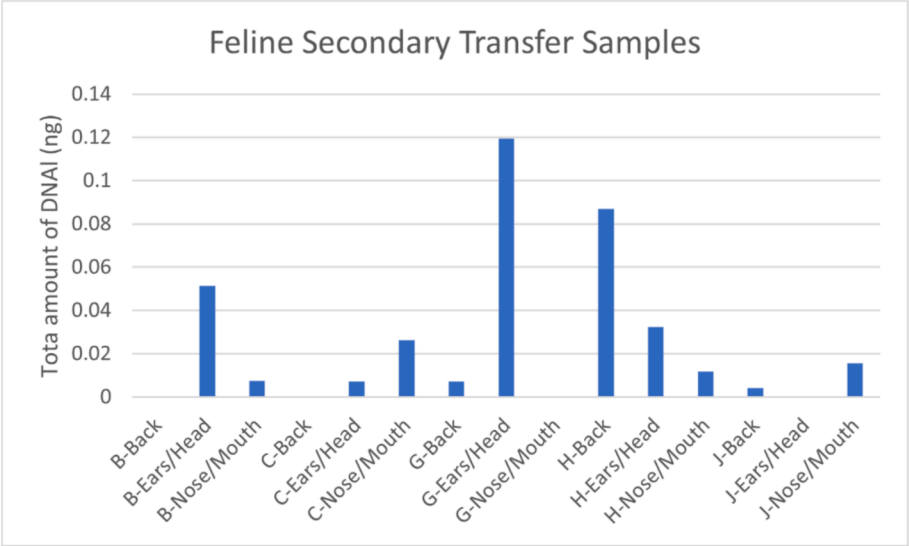

Fig. 2. Total amount of DNA (ng) obtained from the feline secondary transfer samples.

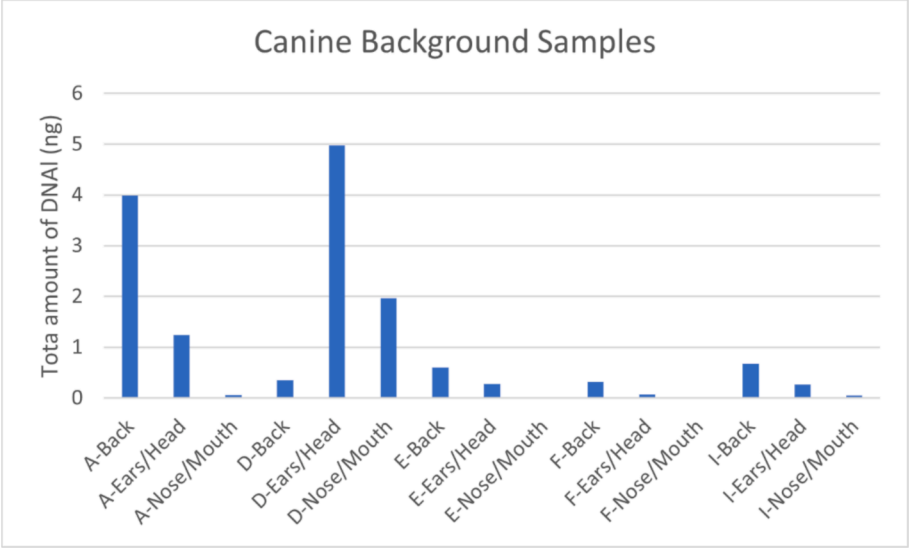

Fig. 3. Total amount of DNA (ng) obtained from the canine background samples.

that yielded DNA amounts that would typically be suitable for amplification, only the direct collection samples were evaluated. No significant patterns were observed, rather variation seems to be attributable to the specific pet (Figs. 5-6) as opposed to the area on the animal that was sampled with a large range in the quantities of DNA obtained by area.

The averages obtained from each animal type by area were also evaluated (Fig. 7). Here a pattern emerges that the nose and mouth area had the lowest average yield for both cats and dogs. This may be due in part to a lower persistence of DNA in that area because of more regular self-cleaning of their faces as well as DNA washed away in the process of eating and drinking. Furthermore, we can again see that the yield from cats is lower than that of dogs, regardless of the area sampled.

Out of 60 samples, approximately 36 % met the cutoff (0.001 ng/ μL) for moving forward for amplification ($N = 22$). To break it down further 53 % of the background DNA feline samples were suitable for amplification ($N = 8$), 13 % of the secondary transfer feline samples met the cutoff for amplification ($N = 2$), 80 % of the background DNA canine samples were suitable for amplification ($N = 12$), and none of the secondary transfer canine samples were suitable for amplification ($N = 0$).

Suitability for comparison was also evaluated. The laboratory requires a minimum of six alleles at non-sex-determining loci to be suitable for comparison. Profiles with less than 6 non-sex-determining loci are deemed inconclusive due to their limited nature. Furthermore, samples that have more than six loci but limited information, i.e. only a few additional loci beyond the cutoff, and are clearly a complex mixture would also be deemed inconclusive for comparison purposes due to the potential for misassignment of NOC, and risk of adventitious matching and unintuitive results observed in internal validation.

Approximately 31 % of all samples were deemed suitable for comparison ($N = 19$). None of the samples from the secondary transfer data set were suitable for comparison ($N = 0$). However, it should be noted that this was with a smooth surface being employed as a vector for transfer. The use of a rough surface such as fabric from clothing may yield different results and would need to be explored separately [9]. Additionally, the manner of contact, presence of body fluids, etc. will also impact the results. Given the smooth surface, these results did vary from a previous study where 35 % of the profiles generated from transfer to a smooth surface from a canine were interpretable. However, this was

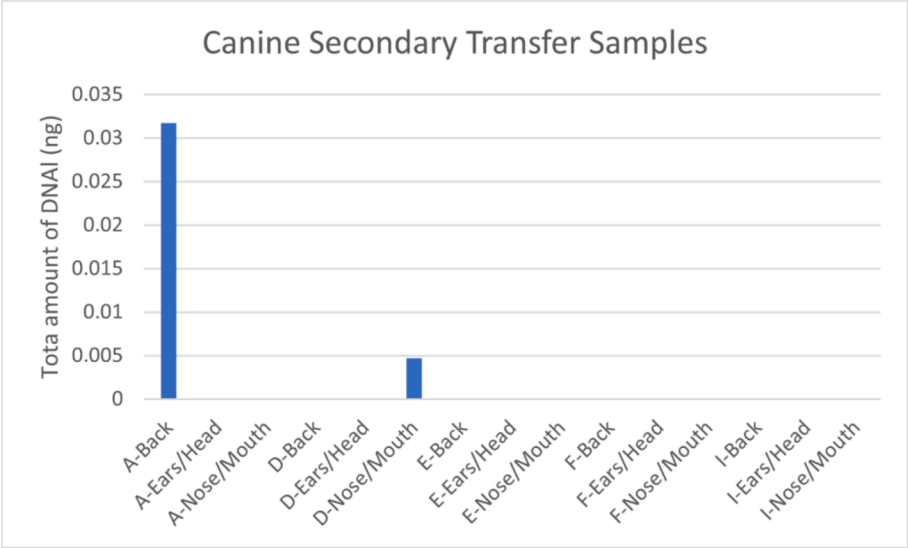

Fig. 4. Total amount of DNA (ng) obtained from the canine secondary transfer samples.

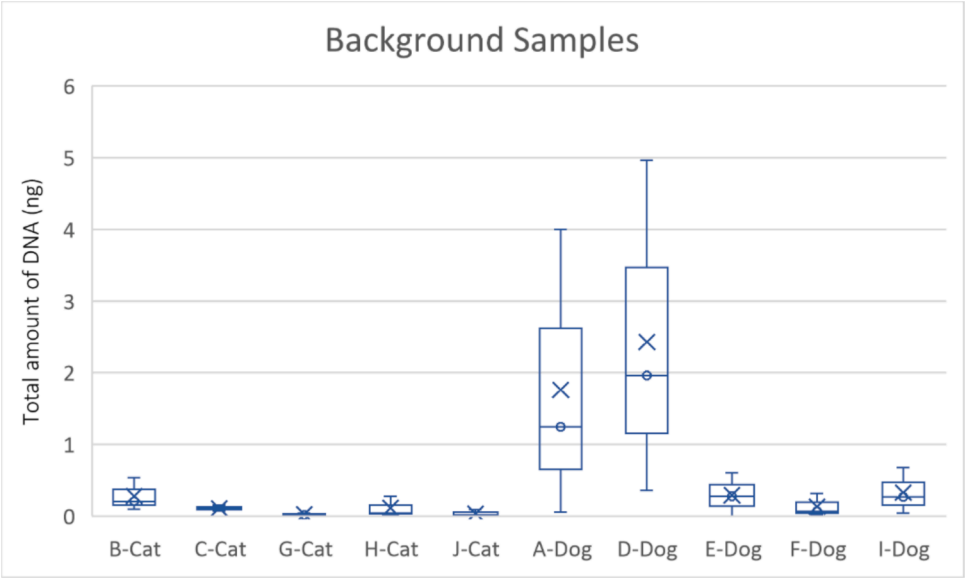

Fig. 5. Total DNA yields by animal from the background samples.

transfer via paws, an area which was not analyzed for this study. The same study also examined transfer of human DNA from dogs to a DNA free glove, which resulted in twenty DNA profiles, 65 % of which were able to be further analyzed [2].

Profile suitability is assessed under several parameters; however, the primary requirement for this study was that a minimum number of 6 non-sex-determining loci must be present for the profile to be deemed suitable for comparison. All 8 samples that were suitable for amplification for the feline samples were also suitable for comparison and 91 % of the 12 samples suitable for amplification for the canine samples were also suitable for comparison ($N = 11$). All samples were compared to the known reference regardless of if an outright exclusion could be made by visual examination.

Number of contributors (NOC) was assessed for each category. For the 8 feline direct samples suitable for comparison 12 % ($N = 1$) of profiles obtained 1 contributor, 62 % ($N = 5$) of profiles obtained 2 contributors, and 13 % ($N = 2$) of profiles obtained 3 contributors. For the 11 canine direct samples suitable for comparison 54 % ($N = 6$) 2 contributors and 45 % ($N = 5$) obtained 3 contributors.

Of the 19 samples suitable for comparison three supported hypothesis 2 (H2) and 16 supported hypothesis 1 (H1), where H1 is the primary pet owner and N-1 unknown individuals, and H2 is N unknown individuals. Of the 16 samples supporting H1, 14 would fall into the 'very strong support' level of support as described by the Scientific Working Group on DNA Analysis Methods (SWGDAM)(Figs. 8-9) [10].

A summary of the data obtained from all samples is shown in Table 1. The estimated proportion of the total DNA is allocated to each contributor in the mixture during STRmix™ deconvolution. In Table 1 the proportion associated with the reported likelihood ratio is shown. Samples that were not suitable for amplification, deemed inconclusive for comparison purposes or that supported the DNA profile under H2 are designated as not applicable (NA) in Table 1 under the proportion column. Of the samples that resulted in inclusionary LR's, the majority of them were assigned a contributor proportion greater than 50 %. For two of the samples from Dog A the primary owner was the minor contributor at 14 % and 18 %, the third direct sample from Dog A supported the

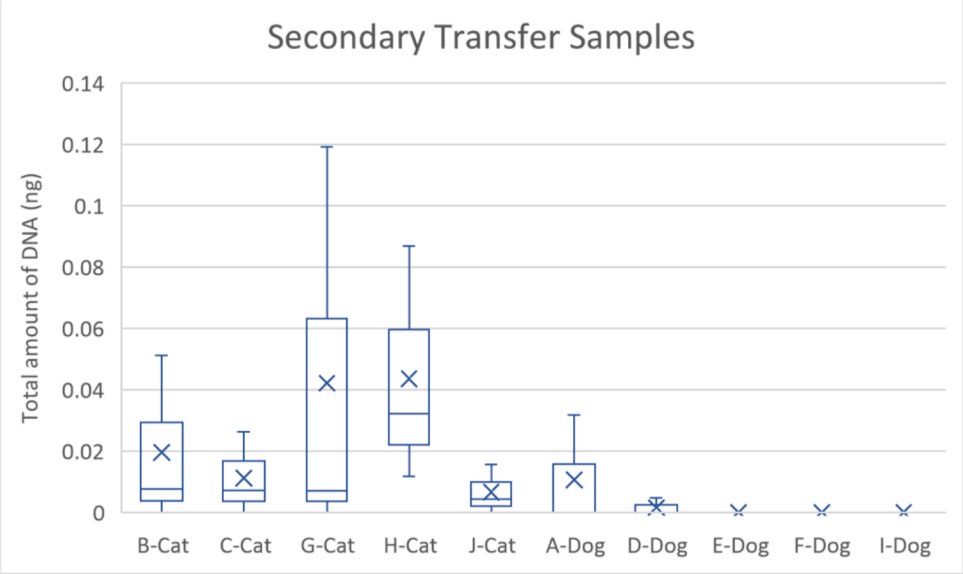

Fig. 6. Total DNA yields by animal from the secondary transfer samples.

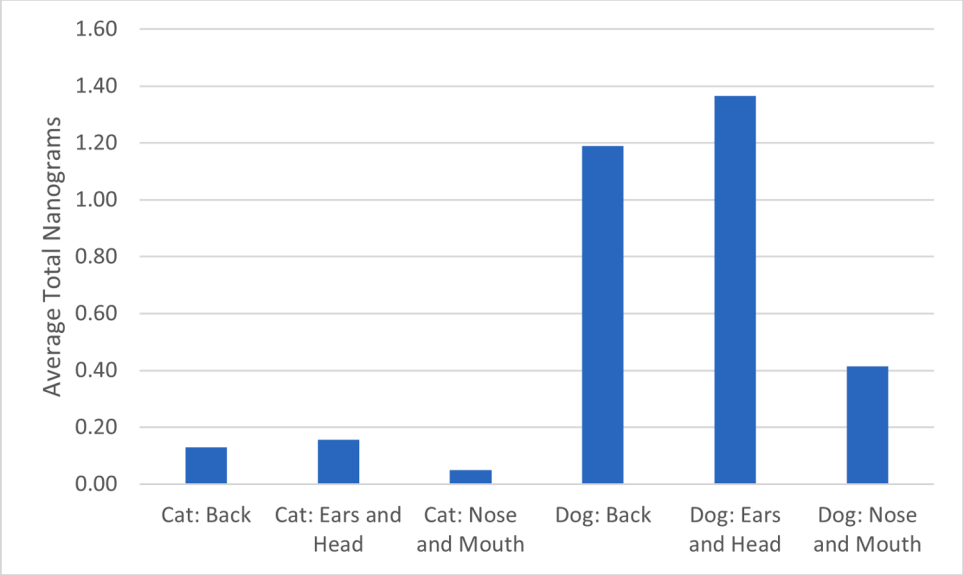

Fig. 7. Average total DNA yield by animal type and area sampled.

exclusion of the primary owner. It should be noted that Dog A comes from a household with two adult humans and the presence of the DNA from the second individual as well as the unknown shedder status of these individuals could account for this disparity.

It should be noted that, beyond sterile gloves, additional personal protective equipment, such as masks and coveralls were not worn by the research participants so it is possible that there could have been researcher contamination.

Amplification artifacts were observed in five of the samples (see Fig. 10 for example), represented by four pets, two cats and two dogs. Non-human polymerase chain reaction (PCR) artifacts are occasionally observed in DNA analysis [11]. Care should be taken in interpreting profiles with potential non-human amplification artifacts as they may not always be apparently artifactual in nature and could size into bins. However, lack of expected stutter peaks, inability to size the peak, presence in known artifact publications, and relative fluorescent unit (RFU) measurements inconsistent with the profile overall can aid the examiner in determining if a peak may be a non-human PCR artifact. Analysis may benefit from reinjection of the sample and/or reamplification of the sample. Additionally, the vendor that distributes the DNA profiling system may be contacted for technical support to inquire whether the artifact has been previously observed by another laboratory.

A summary of all artifacts observed in this particular study are logged in Table 2. Some artifacts were observed more than once, for example, the off-ladder (OL) artifact around 103 base pairs (bp) in size observed at D3S1358 was observed in two cats. This artifact had previously been observed at the laboratory where this research study was conducted. The artifact observed between D19S433 and SE33 around 265 bp, the OL peak around 191 bp at vWA (observed in both cats and a dog), and the OL peak around 270 bp at D7S820 had also been observed at the research laboratory. None of the remaining artifacts have been reported by Promega or the research laboratory.

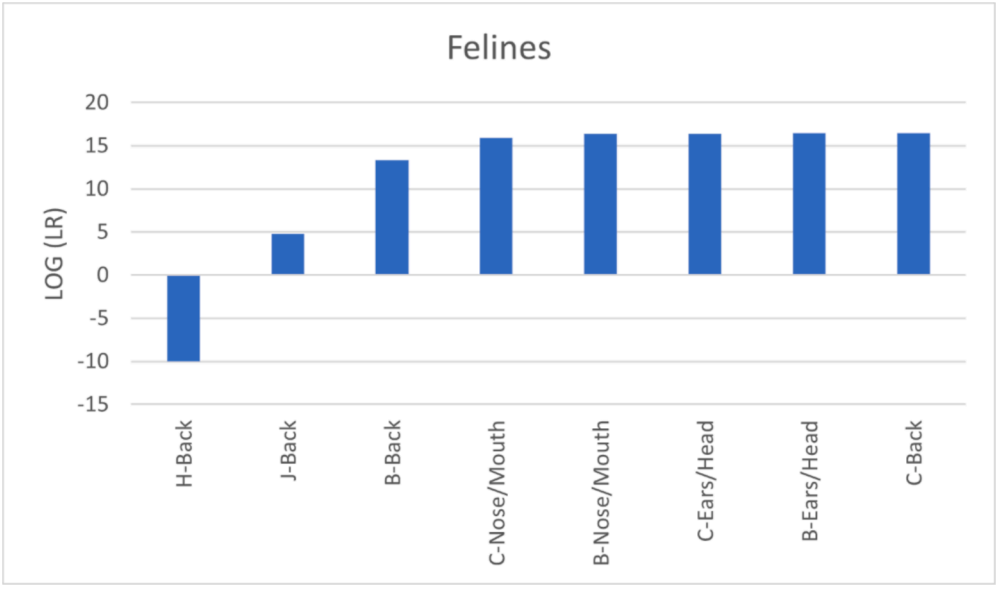

Fig. 8. Log of the Likelihood Ratio obtained for the feline samples suitable for comparison.

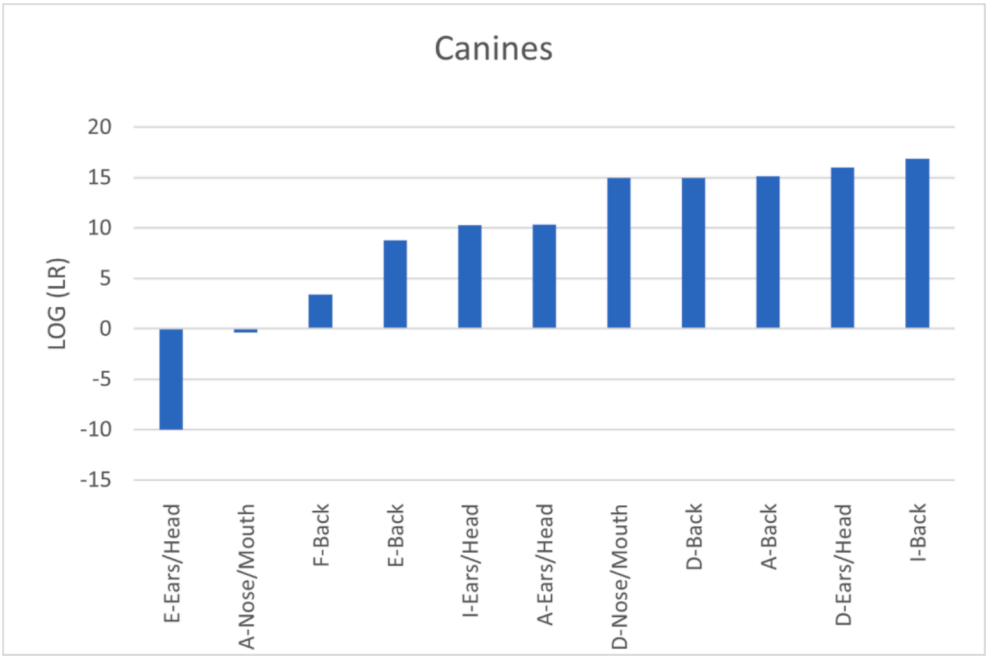

Fig. 9. Log of the Likelihood Ratio obtained for the canine samples suitable for comparison.

4. Conclusions

When considering DNA profiles obtained from evidence, it is imperative to have relevant research data for evaluative reporting. This study demonstrated that as expected, and supporting previous research [1,2], background DNA attributable to a primary pet owner can be obtained directly from canines and felines. Having this additional research to support previous research helps to establish a baseline for the quantities of DNA attributable to a primary pet owner that can be expected to be recovered from the animal in routine circumstances. This study demonstrates that it is possible to obtain LRs that support the DNA profile under the hypothesis that includes the primary owner of a domestic pet when sampling directly from the animal, either canine or feline. However, although human DNA was detected on plastic cards after a secondary transfer scenario was introduced, no profiles suitable for comparison were obtained. Human DNA can be transferred from domestic pet to an item; however, given the sensitivity of current testing it is unlikely that it will result in a DNA profile suitable for comparison under the circumstances outlined in this experiment. The DNA analyst should take into consideration, the type of item in question, the type of pet in question, the behavior of the pet in the household, and whether any mitigating circumstances may have occurred, using available relevant literature. For example, the presence of wet body fluids which may change the hypotheses that need to be considered when evaluating the DNA results for evaluative reporting. Additional research may benefit from the evaluation of additional item types, including a second human to pat the animal, and expansion into other domestic pet types such as birds, lizards, ferrets, gerbils, etc. may also be beneficial.

Table 1
Summary of all results (NOC – number of contributors, ng – nanograms, alleles – total alleles observed).

Pet ID	Pet Type	Area	Transfer	Total Human	Total Male	Alleles	NOC	LR	LOG(LR)	Proportion
A	Dog	Nose/Mouth	Background	0.05	0.05	35	2	4.44E-01	−0.35	NA
A	Dog	Ears/Head	Background	1.24	1.07	97	3	2.11E + 10	10.33	14 %
A	Dog	Back	Background	4.00	2.85	88	3	1.39E + 15	15.14	18 %
A	Dog	Back	Secondary	0.03	0.01	3	NA	NA	NA	NA
A	Dog	Nose/Mouth	Secondary	0.00	0.00	0	NA	NA	NA	NA
A	Dog	Ears/Head	Secondary	0.00	0.00	0	NA	NA	NA	NA
B	Cat	Back	Background	0.21	0.10	83	3	2.31E + 13	13.36	53 %
B	Cat	Nose/Mouth	Background	0.10	0.02	53	2	2.33E + 16	16.37	86 %
B	Cat	Ears/Head	Background	0.54	0.02	59	3	2.86E + 16	16.46	95 %
B	Cat	Back	Secondary	0.00	0.00	1	NA	NA	NA	NA
B	Cat	Nose/Mouth	Secondary	0.01	0.00	0	NA	NA	NA	NA
B	Cat	Ears/Head	Secondary	0.05	0.00	2	NA	NA	NA	NA
C	Cat	Nose/Mouth	Background	0.11	0.01	50	2	8.87E + 15	15.95	85 %
C	Cat	Ears/Head	Background	0.15	0.02	53	2	2.49E + 16	16.40	89 %
C	Cat	Back	Background	0.07	0.00	41	2	3.03E + 16	16.48	90 %
C	Cat	Back	Secondary	0.00	0.00	0	NA	NA	NA	NA
C	Cat	Nose/Mouth	Secondary	0.03	0.01	1	NA	NA	NA	NA
C	Cat	Ears/Head	Secondary	0.01	0.00	0	NA	NA	NA	NA
D	Dog	Nose/Mouth	Background	1.96	0.87	91	3	8.57E + 14	14.93	59 %
D	Dog	Back	Background	0.35	0.06	79	3	8.83E + 14	14.95	61 %
D	Dog	Ears/Head	Background	4.97	1.50	90	3	9.78E + 15	15.99	67 %
D	Dog	Nose/Mouth	Secondary	0.00	0.00	0	NA	NA	NA	NA
D	Dog	Ears/Head	Secondary	0.00	0.00	0	NA	NA	NA	NA
D	Dog	Back	Secondary	0.00	0.00	0	NA	NA	NA	NA
E	Dog	Ears/Head	Background	0.27	0.03	49	2	0	−10.00	NA
E	Dog	Nose/Mouth	Background	0.00	0.00	0	NA	NA	NA	NA
E	Dog	Back	Background	0.60	0.06	58	2	6.01E + 08	8.78	65 %
E	Dog	Back	Secondary	0.00	0.00	0	NA	NA	NA	NA
E	Dog	Nose/Mouth	Secondary	0.00	0.00	0	NA	NA	NA	NA
E	Dog	Ears/Head	Secondary	0.00	0.00	1	NA	NA	NA	NA
F	Dog	Nose/Mouth	Background	0.01	0.00	2	NA	NA	NA	NA
F	Dog	Ears/Head	Background	0.07	0.00	7	NA	NA	NA	NA
F	Dog	Back	Background	0.32	0.05	44	2	2.49E + 03	3.40	60 %
F	Dog	Back	Secondary	0.00	0.00	1	NA	NA	NA	NA
F	Dog	Nose/Mouth	Secondary	0.00	0.00	0	NA	NA	NA	NA
F	Dog	Ears/Head	Secondary	0.00	0.00	0	NA	NA	NA	NA
G	Cat	Back	Background	0.02	0.00	0	NA	NA	NA	NA
G	Cat	Nose/Mouth	Background	0.01	0.00	1	NA	NA	NA	NA
G	Cat	Ears/Head	Background	0.04	0.00	8	NA	NA	NA	NA
G	Cat	Back	Secondary	0.01	0.00	0	NA	NA	NA	NA
G	Cat	Nose/Mouth	Secondary	0.00	0.00	0	NA	NA	NA	NA
G	Cat	Ears/Head	Secondary	0.12	0.00	6	NA	NA	NA	NA
H	Cat	Back	Background	0.27	0.00	23	1	0	−10.00	NA
H	Cat	Nose/Mouth	Background	0.04	0.00	18	NA	NA	NA	NA
H	Cat	Ears/Head	Background	0.02	0.00	0	NA	NA	NA	NA
H	Cat	Back	Secondary	0.09	0.00	12	NA	NA	NA	NA
H	Cat	Nose/Mouth	Secondary	0.01	0.00	1	NA	NA	NA	NA
H	Cat	Ears/Head	Secondary	0.03	0.01	1	NA	NA	NA	NA
I	Dog	Nose/Mouth	Background	0.04	0.00	5	NA	NA	NA	NA
I	Dog	Ears/Head	Background	0.27	0.03	31	2	1.78E + 10	10.25	64 %
I	Dog	Back	Background	0.68	0.01	64	2	7.34E + 16	16.87	87 %
I	Dog	Back	Secondary	0.00	0.00	0	NA	NA	NA	NA
I	Dog	Nose/Mouth	Secondary	0.00	0.00	0	NA	NA	NA	NA
I	Dog	Ears/Head	Secondary	0.00	0.00	0	NA	NA	NA	NA
J	Cat	Nose/Mouth	Background	0.00	0.00	0	NA	NA	NA	NA
J	Cat	Ears/Head	Background	0.02	0.00	0	NA	NA	NA	NA
J	Cat	Back	Background	0.09	0.00	24	2	6.70E + 04	4.83	57 %
J	Cat	Back	Secondary	0.00	0.00	0	NA	NA	NA	NA
J	Cat	Nose/Mouth	Secondary	0.02	0.00	0	NA	NA	NA	NA
J	Cat	Ears/Head	Secondary	0.00	0.00	0	NA	NA	NA	NA

Ethics Statement

All authors have seen and approved this manuscript. Permission to publish this manuscript was not required from the author's institutions. Human and Animal Research Ethics Clearance for this study was obtained from Griffith University (GU Ref No: 2022/298).

CRediT authorship contribution statement

Rachel Oefelein: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Project administration, Visualization, Writing – original draft. **Sarah Cresswell:** Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing. **Carney Matheson:** Project administration, Supervision, Writing – review & editing.

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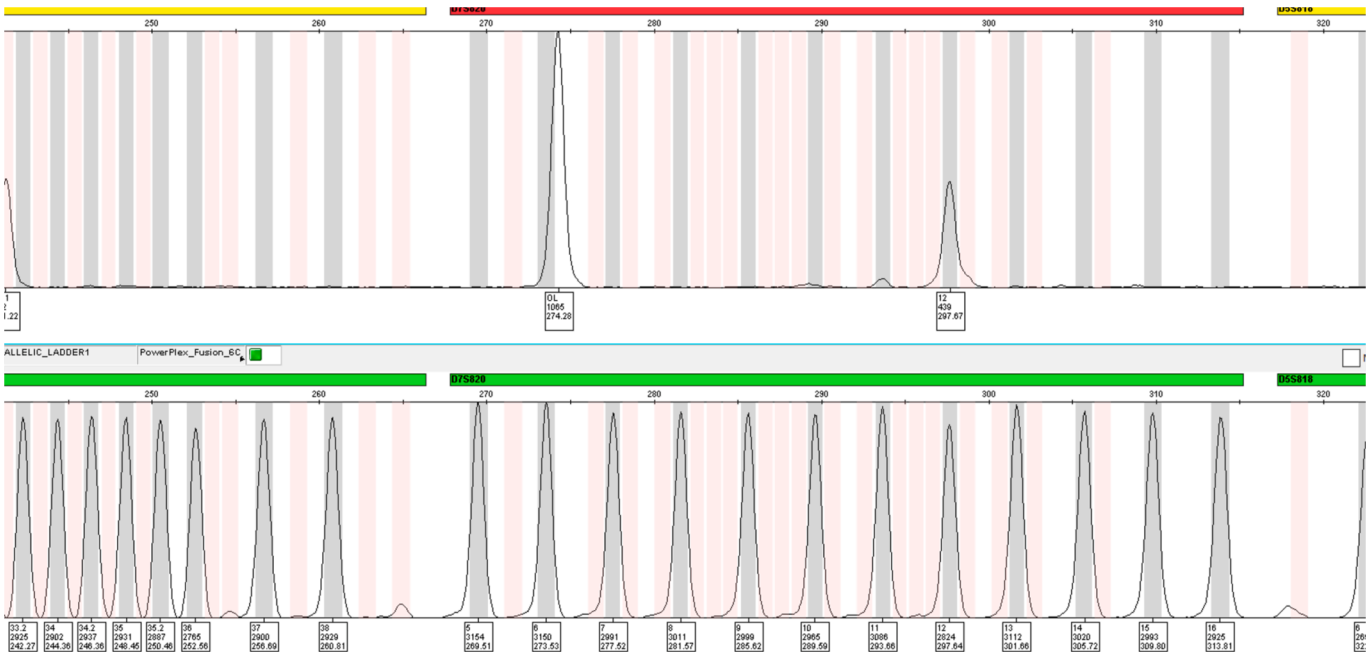

Fig. 10. Non-human PCR artifact observed at the D7S820 locus for this research that was also previously observed and reported by the laboratory conducting this research.

Table 2
Summary of artifacts observed. (OL – off-ladder, OMR – outside marker range, bp – base pair).

Pet	Dog/ Cat	Area	Dye	Locus	Artifact Size (~bp)	Allele Call
A	Dog	Back	Red	D8S119	101.9	OL
A	Dog	Head/ Ears	Yellow	D7S820	270.7	OL
C	Cat	Back	Blue	D3S1358	102.72	OL
C	Cat	Back	Blue	D3S1358	116.49	OL
C	Cat	Back	Blue	D1S1656	167.51	OL
C	Cat	Back	Green	D18S51	172.22	OL
C	Cat	Back	Green	NA	306.8	OMR
C	Cat	Back	Green	Penta D	383.33	OL
C	Cat	Back	Yellow	vWA	191.09	OL
C	Cat	Back	Yellow	D7S820	274.11	OL
C	Cat	Back	Yellow	TPOX	440.95	OL
C	Cat	Back	Red	NA	264.89	OMR
D	Dog	Nose/ mouth	Blue	D1S1656	170.54	12
D	Dog	Nose/ mouth	Blue	D1S1656	171.56	OL
D	Dog	Nose/ mouth	Yellow	vWA	190.87	OL
H	Cat	Nose/ mouth	Blue	D3S1358	102.7	OL
H	Cat	Nose/ mouth	Blue	D1S1656	183.75	OL
H	Cat	Nose/ mouth	Blue	D13S317	349.52	OL
H	Cat	Nose/ mouth	Green	D18S51	172.19	OL
H	Cat	Nose/ mouth	Yellow	vWA	191.03	OL

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Author Rachel H. Oefelein works at DNA Labs International and provides expert witness testimony in court for both DNA analysis and Serology.

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