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Paws for a moment: Investigation of bi-directional transfer of human DNA during a short human–dog interaction and subsequent indirect transfers

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ABSTRACT

DNA transfer events have been well researched in the context of commonly found items at crime scenes. However, whilst animals are a common feature of most households, transfer events involving companion animals have been understudied. Recent research has shown that dogs and cats are a reservoir of human DNA that can transfer to a hand or sterile object after a short contact. It is now of interest to explore how and where this DNA on dogs can transfer during normal human-animal interactions. In order to assess this aspect of DNA transfer, 5 dogs were paired with 5 visitors that were unknown to one another. The dogs were contacted by the visitor for 5 min and the first 5 items contacted immediately were sampled. Additionally, the first 3 items the visitor touched on return home, a number of pre-determined items they were instructed to touch, and both hands, were also targeted. Finally, the dog and the items that the animal contacted in an hour post contact were also analysed.

The results showed that DNA of the visitor or visitor's housemate persisted on the dog up to an hour later in 50 % of the samples but was not detected on any of the items an animal contacted. Dog owner's DNA transferred from the dog to the visitor and visitor related items and surfaces, including the car and the house, in 31 % of the samples. These results provide a valuable insight for forensic investigators on the potential origins of DNA found at a crime scene and also add to the body of research indicating that companion animals may be used as evidence to identify who had been in contact with a dog.

1. Introduction

Several studies have investigated the quantity and quality of transferred touch DNA, whether deposited directly or indirectly [1–7], and the relative impact of substrates [8–13], manner of contact [14] and aspects of shedder status [15–17] on these deposits. Similarly, studies have explored persistence in different environments [18,19] and after subsequent object use [5,9,20]. However, few resources have been directed towards the study of human DNA transfer, persistence, prevalence and recovery (DNA-TPPR) in scenarios involving pets. Monkman et al. [21,22] demonstrated that domestic dogs and cats accumulate human DNA, typically from their owners, and that this DNA can be readily transferred during contact.

Household pet dogs are more likely to be a victim of a criminal activity than other animals within the home (e.g. physically abused or stolen) [23], may be bystanders during an offence involving an incidental interaction with an offender (e.g. where contact was made to calm, move or repel a dog), or may disrupt intruders at a crime scene. These interactions may result in potential target sites to acquire DNA of a person of interest (POI) to assist forensic investigations and/or potential removal, redistribution and/or contamination of DNA evidence, complicating DNA profile interpretations and activity level evaluations. Thus, investigating the possibility, extent, and persistence of human DNA transfer to and from dogs with contact is of relevance to assist in DNA interpretation where animals are present during evidence collection.

Limited research has been done to investigate how animals interact with criminals in intruder-based scenarios and what areas on an animal should hence be sampled. Therefore, for the current study, we used results from Monkman et al. [24] to inform sampling locations. In the study, types of interactions an intruder may have with a dog were assessed by analysing publicly available video recordings on YouTube,

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Google and TikTok. These recordings consisted of guests entering the home and making contact with the household dog, with particular focus on where the contact was made e.g. the head, sides or back of the animal. The videos showed that the head and back were most contacted areas and these areas were then targeted in this study.

The presence and amount of DNA transfer to and from an animal and a visitor, after a 5-minute interaction, was investigated in the present study. Additionally, subsequent transfer of the DNA picked up from an animal to the visitor's car, hands and house was also assessed. Conversely, persistence of visitor's DNA on touched areas of the dog and on surfaces with which they had subsequent contact were also investigated. Ultimately, this research aims to expand our understanding of how readily DNA can be transferred between a dog, a visitor and subsequently contacted surfaces, highlighting the implications of having pets at crime scenes.

2. Methods

2.1. Ethics

All samples were collected with informed consent and both Animal and Human Ethics approvals Flinders University Human Ethics Committee approval (5206) and the Flinders University Animal Ethics Committee approval (5202).

2.2. Experimental setup

Five dogs of different breeds (Supplementary data 1) were recruited from five households, based on their perceived friendliness, as assessed by their owners. Five individual people not belonging to the same household as the dog owners, deemed the "visitor," were then recruited to pat and play with one dog each. The paired dog owners and visitors had not previously been in each other's house or car.

During the experiment, a visitor drove to the dog's household and entered the porch area with explicit instructions to make contact with the dog only. A 2.5 m DNA-free sheet was placed on the ground to ensure that the visitor did not accumulate any DNA from the household entry way. The dog was leashed by their owner and walked to the porch entrance, where the visitor interacted physically (unscripted) with the dog for five minutes, by patting and/or playing with them. Once the interaction was completed, the visitor left the porch, returned to their car and drove home. The researcher sat in the backseat of the visitor's car wearing PPE, on the passenger side (opposite to the visitor), to observe and record what surfaces the visitor contacted throughout the drive. The researcher took DNA samples from the first five surfaces the visitor contacted, prior to home arrival, which were predominantly surfaces in the car including outside door handle, steering wheel, gear stick, inside door handle, hand brake, inside door handle, indicator and phone (Fig. 1, 2a, Supplementary data 1, tab 2). In one experiment, the visitor did not contact five separate surfaces before entering their house (dog 2). None of the five items touched after leaving the experiment included a seat belt (that was usually item 6–10 the sequence of contacts made).

Similarly, on arrival, the first three items the visitor touched were also sampled (Fig. 1, 2b). Approximately 10–20 min after arriving home, or 40 min after the staged interaction with the dog, the visitor was instructed to make a cup of tea using their kettle and a mug. After making the tea, the handles of the kettle and the mug (Fig. 1, 2c) (predetermined items in all 5 experiments), as well as the hands of the visitor, were sampled (Fig. 1, 2d). None of the visitor items were precleaned prior to experiments.

During this time, the owner of the dog was instructed to refrain from touching their dog and asked to keep the dog inside, recording and photographing its location and subsequent contacts, until the researcher returned approximately one hour later to take the samples. The two areas in which the dogs spent time post-interaction were also sampled, when available (Fig. 1, 3a). Four separate areas (measuring approximately 10 cm x 5 cm) of fur were then sampled from the dog: the left side of the body, the right side of the body, the top of the head, and the top of the back (Fig. 1, 3b). None of the items contacted by the visitor and dog were cleaned prior to sampling, in order to simulate a realistic casework scenario. Information regarding the breed of dogs, hair length and general behaviour of the dogs can be found in Supplementary data 2.

PPE was worn by the researcher at all times (gloves, mask, gown, hairnet). Reference samples were collected from each dog owner, their adult house co-habitants, each visitor, and the researcher. Further, information regarding the visitor's living conditions, number of housemates, and frequency of visitors was self-reported in a questionnaire (Supplementary data 2).

2.3. Sample collection and processing

All experimental samples were taken using a wet-dry double swabbing technique using viscose swabs (Forensic Swab L, Sarstedt) and sterile water [25]. DNA was extracted, quantified, amplified and analysed using DNA IQ™ (Promega), Quantifiler Trio™ (Applied Biosystems) and PowerPlex® 21 (Promega) kits, respectively, as per manufacturer recommendations. The maximum input template DNA for PCR was 0.5 ng in a 25 µl reaction (30 cycles). When the DNA concentration of the extract was <0.033 ng/µl, 15 µl of a 60 µl extraction volume was used. PCR products were run on a 3500xL Genetic Analyser (ThermoFisher) (1.2kv/24 sec) and typed using GeneMapper™ IDx Software (v1.6, ThermoFisher) with a baseline threshold of 175 RFU. The total amount of DNA in the sample was calculated by multiplying the DNA concentration by the extract volume (60 µl).

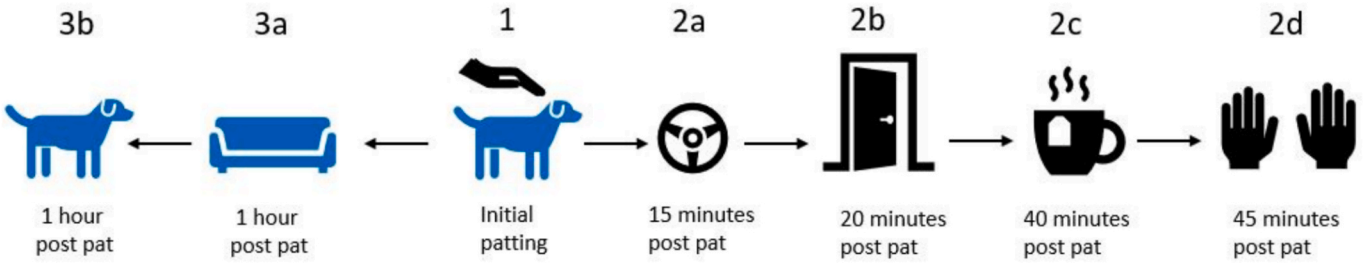

Fig. 1. Experimental design and sample targeting: 1- The dog was patted. 2a- Samples taken from areas of the visitor's car the visitor touched immediately after patting and on their way home. 2b- Samples taken from the first three items the visitor touched that were part of or in their house. 2c- Approximately 40 min after the interaction with the dog the visitor prepared a cup of tea and samples were taken from the mug and kettle handles. 2d- Sample taken from the visitor's left and right hands approximately 45 min after patting. 3a- Samples taken from the items the dog contacted in the hour post patting. 3b- samples taken from the head, back and sides of the dog.

2.4. DNA profile interpretation

The number of contributors in a sample was determined using the maximum allele count and allelic peak height balance. The statistical software STRmix™ (v2.9, ESR and FSSA) was used for mixture deconvolution and likelihood ratio calculations (see Supplementary data 1). Profiles with no allelic peaks were categorised as providing no profile (NP). Profiles were determined as having drop out if no alleles were observed in at least one locus. Locus rather than allele drop out was used to designate partial profiles as most of the samples obtained were mixtures where allele sharing can mask drop out occurrences. STRmix™ mixture proportions were used to assign major (≥70 %) and minor (<70 %) contributors within a mixed sample. Further, in mixed profiles where there was no major contributor, majority (most DNA in the sample) and minority contributors were identified. A "person of interest" was considered detected when the likelihood ratios (LRs) generated by STRmix™ were equal to or greater than 100. All inclusionary LRs below 100 were considered to be adventitious and assigned an "unknown donor" designation. Samples generating profiles with inclusionary LRs below 100 can be found in Supplementary data 1 (these samples are highlighted in blue). STRmix™ v2.9 (ESR and FSSA) was also used to complete mixture-to-mixture comparisons (LR calculations without θ) and an LR of 100 threshold was set for possible matches. This was done for the purpose of investigating common unknown donors. Statistical analysis was undertaken to investigate the possible relationship between the amounts of DNA from different areas on the body of the dog and the different areas sampled (Kruskal-Wallis test; ≤0.05) and the amounts of DNA detected on different body areas vs the visitor detection (based on inclusionary LRs ≥ 100) (Chi squared test, ≤0.05).

3. Results

3.1. Samples from the dog and dog contacted surfaces post-interaction with the visitor

An hour after contact, the total amount of human DNA on the designated areas of the dog (n = 20) ranged from 0.13-3.06 ng (av.1.4 ng) (Table 1). Note, during the one-hour period where owners were asked not to touch their dogs, two dogs (dogs 2 and 4) were patted on the back between 1–3 times and one dog (dog 5) was patted on its side and head on three occasions (Supplementary data 1). All dogs had quantifiable human DNA recovered from the head, back and sides of the body. There was no significant difference in the amounts of DNA recovered from the different areas on the body of the dog (p = 0.228).

The items the dogs came into contact with post-visit (n = 6) had total quantifiable DNA ranging between 1.14 and 4.62 ng (av. 2.3 ng) (Table 1; Supplementary data 1). One dog did not sit down at all, two dogs sat down in a single location, and the remaining two dogs spent time in two different areas prior to sampling. The minimum number of contributors ranged from 2 to 4 (av. 3) from both the dogs and associated items. At least one owner was detected on all the items sampled, with two owners detected in 83 % of samples (LR ranging 3.8 x 10^3–2.2 x 10^{25}, av. 2.1 x 10^{24}).

Table 1
Total amount of human DNA (ng) recovered from the dogs and areas contacted by the dogs post-visitor interaction.

	Dog				
Sampled area/item	1	2*	3*	4	5*
Dog's head	0.66	2.04	0.48	0.72	0.48
Dog's back	1.20	1.5	3.00	1.08	3.06
Dog's left side	1.20	2.58	0.96	0.48	1.74
Dog's right side	0.60	2.10	0.13	0.48	2.64
1st item dog touched after visitor	1.14 *Couch blanket*	4.62 *Dog bed*	–	1.92 *Bed 1 office*	1.44 *Dog bed*
2nd item dog touched after visitor	1.44 *Couch arm*	–	–	3.42 *Bed 2 lounge*	–

*Dogs did not sit down or stayed in one place only.

Table 2 summarises the number of times transfer was observed from the visitor to the dog and the items contacted by the animal after the interaction, and from the dog to the visitor and the associated items they contacted, including information on the LR ranges and DNA quantities detected for each sample type. The visitor's or visitor related person's (housemate of one of the visitors) DNA was transferred to the dog in 50 % (10 of 20) of the samples. The visitor was never detected as the major contributor but was the minor or minority contributor in 35 % of the dog samples (7 of 20, LR ranging from 3.0 x 10^2–2.7 x 10^{10}, av. 4.8 x 10^9). There was no statistical significance (p = 0.243) between the different body areas of the dog sampled and the presence/absence of the visitor's DNA. Further, a visitor's housemate was detected in 15 % of the samples (3 of 20), all from the same dog (dog 3), although the visitor themself was not detected. This housemate was a major contributor in one sample (LR = 2.8 x 10^{16}) and a minor contributor in two samples (LR = 5.7 x 10^{11} and LR = 8.1 x 10^{11}). The visitor or someone from the visitor's household was more frequently detected on dogs 1 and 3 relative to the other dogs (Supplementary data 1).

At least one of the of the dog's owners was detected in every sample, as either the major (35 % of the samples; 7 of 20 samples), majority (60 % of the samples; 12 of 20 samples) or as the minor/minority contributors (60 % of the samples; 12 of 20 samples) (LRs ranging from (8.2 x 10^3–3.4 x 10^{25}, av. 4.11 x 10^{24}). In half of the samples, both owners were detected. On a daily basis, the dogs spent between 30 min and 10 h in contact with their owners (Supplementary data 1). Dogs 2 and 5 spent the most time in direct contact with their owners, averaging 4.5 and 7.5 h per day, respectively, and had larger quantities of owner DNA recovered (av. 1.02 ng; combining all owner' contributions) when compared to the other dogs (av. 0.44 ng; combining all owner contributions). Interestingly, these increased amounts of owners' DNA did not appear to influence the amounts of visitors' DNA detected.

Unknown donors were detected as a minor contributor in 80 % of the samples (16 of 20 samples) and each dog had accumulated DNA from an unknown person in at least one body area. Unknown donors were not detected as the major or majority contributors. Opportunities for unknown DNA transfer were investigated using the self-reported questionnaire data. For three dogs (dogs 2, 4 and 5), a guest had visited the house in the 12–24 h prior to sampling, while the other two did not have visitors for at least a week prior to sampling. Further, two dogs had been walked in the 24-hours prior to sampling. During the walk, one of the dogs was patted by a passerby (dog 4) and the other dog spent time in the children's playground (dog 1).

Mixture-to-mixture comparisons identified common unknown donors in several body samples for each of the four dogs where multiple samples had unknown DNA (dogs 1, 2, 4 and 5), as well as the surfaces contacted by each dog post-interaction (Supplementary data 1). Notably, in one instance, unknown donor's DNA, that was detected on the left side, back and both items the dog sat on after sampling, was also found on the visitor's outside car handle (dog 1) (Supplementary data 1). Interestingly, this unknown DNA was not detected on the hands of the visitor. Additionally, in another instance, unknown DNA detected on the left hand of the visitor was also detected on the steering wheel, the phone, and the kettle (dog 3). Finally, another unknown contributor was detected on the right side and head of the dog and dog's bed as well as the non-dominant hand of the visitor (dog 2) (Supplementary data 1).

3.2. Samples collected from visitor contacted surfaces

3.2.1. First five surfaces touched immediately after contact with the dog

Of the five items touched by visitors immediately after interacting with their designated dogs, before arriving home (n = 24) (one of the visitors only contacted four items), the most commonly contacted ones were the steering wheel, the gear stick and the outside car door handle of

Table 2
The number of times DNA transfer was observed from the visitor (both visitor's and other's DNA) to the dog and the associated items that the animal contacted, and from the dog to the visitor and the associated items they contacted, including information on the ranges of DNA quantities and LRs detected for each sample type (See Supplementary data 1 for details per sample).

Samples	Transfer from the visitor detected	Transfer from the owner detected	Range of visitor's DNA and LR's (ng)	(LR)	Range of owner's DNA and LRs (ng)	(LR)
Dog (3b) (n = 20)	7 (10[a])	20	0.10–0.26 (av. 0.18)	3.0 x 10^2–2.7 x 10^{10} (av. 4.8 x 10^9)	0.02–2.11 (av. 0.71)	8.2 x 10^3–3.0 x 10^{25} (av. 4.1 x 10^{24})
Dog's items (3a) (n = 6)	0	6	NA	NA	0.36–3.79 (av. 1.09)	3.8 x 10^3–2.2 x 10^{25} (av. 2.1 x 10^{24})
First 5 items in the car (2a) (n = 24)	24	8(10[b])	0.12–17.59 (av. 3.47)	1.1 x 10^3–8.9 x 10^{25} (av. 5.6 x 10^{24})	0.09–3.56 (av. 0.76)	2.5 x 10^3–2.6 x 10^{19} (av. 2.6 x 10^{18})
First 3 items in the house (2b) (n = 15)	14	0	0.17–10.56 (av. 2.52)	2.8 x 10^9–3.7 x 10^{25} (av. 3.2 x 10^{24})	NA	NA
Kettle and mug (2c) (n = 10)	8	3	0.04–6.29 (av. 2.04)	4.4 x 10^{10}–8.6 x 10^{25} (av. 1.1 x 10^{24})	0.07–0.23 (av. 0.14)	5.4 x 10^3–2.5 x 10^{14} (av. 8.3 x 10^{13})
Visitor's hands (2d) (n = 10)	10	8(10[b])	1.43–7.76 (av. 3.22)	9.1 x 10^{18}–9.6 x 10^{25} (av. 1.5 x 10^{25})	0.12–3.46 (av. 1.03)	8.2 x 10^3–4.5 x 10^{16} (av. 7.9 x 10^{15})

[a]For one dog (Dog 3), the visitor was not detected on sampled areas of the dog, but one of their housemates was (3x). When this housemate is included as an occasion of visitor derived DNA being detected, the number of occasions becomes 10. The quantities and LRs for these three occasions were not included in the table, however, if they were the quantity range would become 0.08–0.69 ng (av.0.48) and the LR range 5.7 x 10^{11}–2.8 x 10^{16} (av. 9.2 x ^{15}).
[b]In some instances, two owners were detected in a single sample. As we are primarily interested in the occurrence of detecting any owners from a household, the presence of one or more owners was counted here as a single occurrence. However, in brackets we provide the overall number of occasions an owner is detected. Note, the quantities and LR's reported in the table are inclusive of all 10 occasions.

their cars. Other items touched included inside car door handle, handbrake, phone and indicator (Supplementary data 1 (the first five items from car)). The total quantity of DNA recovered from these items ranged from 0.12-22.26 ng (av. 4.25 ng), and a DNA profile was able to be generated from all surfaces.

The visitor was detected on all of the surfaces tested either as a major or majority contributor (LRs ranging from 1.1 x 10^3 to 8.9 x 10^{25}, av. 5.6 x 10^{24}). The number of contributors on these surface samples ranged from 1 to 4 (av. 3) (Supplementary data 1). The dog owner's DNA was detected in 33 % (8 of 24 samples) of the samples. There were two occasions where two owners were found in the same sample (LR for the 10 owners detected ranged from 2.5 x 10^3–2.6 x 10^{19}, av. 2.6 x 10^{18}) and these samples were associated with dogs 2–5. The owner of dog 1 was not detected in the car. Dog owners were detected on the steering wheel (3x), outside car door handle (2x), gear stick (2x) and hand brake (1x). In 58 % of the samples (14 of 24 samples) an unknown donor was detected as a minor contributor.

3.2.2. *First three items contacted by visitor on return to their house*

The outside or inside door handle was the first of the three items contacted by each visitor, and the fridge handle was the second item on three occasions. Other items contacted included a kitchen tap, a laptop and mouse, a lid of a jar and a cheese packet. DNA quantities recovered ranged from 0-13.32 ng (av. 3.54 ng) with 93 % of samples (14 of 15 samples) providing detectable quantities from which profiles were able to be generated. Of the 14 samples that provided a DNA profile, the visitor was detected as a major or majority contributor in 71 % of samples (LR ranging 1.8 x 10^{12} to 2.5 x 10^{25}, av. 4.4 x 10^{24}) and as a minor or minority contributor in 21 % of samples (LR ranging 7.3 x 10^{12} to 5.2 x 10^{17}, av. 1.3 x 10^{17}). The number of contributors in the profiles generated ranged from 2 to 3 (av. 2) (Supplementary data 1). One of the visitor's housemates was detected as the major or majority contributor in 14 % of the samples (2 of 14 samples). In 71 % of the samples (10 of 14 samples), an unknown contributor was detected as a minor contributor. In one of the 10 samples with unknown donors, an additional, second major contributor unknown donor was present. The dog owner was excluded from all of these samples. In one sample, an unknown contributor was the major donor with the visitor as the minor contributor.

3.2.3. *The kettle and mug used approximately 40 min after animal contact*

Between arriving home and being instructed to make a cup of tea, the visitors touched objects that were not sampled, such as food items and cutlery, likely both depositing and collecting DNA present on these surfaces. Total DNA from the mugs and the kettles ranged from 0.12 to 4.74 ng (av. 1.37 ng) and 0.42–6.42 ng (av. 2.70 ng) respectively. The number of contributors for these samples was 2–3 (av. 3).

The visitor was the major or majority contributor in 60 % of these samples (6 of 10 samples) (LR ranging 1.1 x 10^{17}–8.6 x 10^{25}, av. 1.6 x 10^{25}) and the minority contributor in 20 % of the samples (LR of 4.4 x 10^{10} and 1.9 x 10^{12}). The visitor was excluded in 20 % (2 of 10 samples). Unknown donor's DNA was also detected in 60 % of samples (6 of 10 samples), always as a minor contributor. A dog's owner was detected in 30 % of samples (3 of 10 samples) (from dog's 2 and 3), two from a kettle and one from a mug. In two of these samples the owner was the majority contributor in mixtures with housemate and unknown DNA (LRs of 8.5 x 10^3 and 2.5 x 10^{14}). In the third sample, the owner was a minor contributor (LR 5.4 x 10^3) in combination with a visitor and a housemate. Of note, these owners were also detected on the hands of the visitors (see Sections 3.2.4 and 3.2.1 and Supplementary data 1) suggesting multi-step transfer events.

3.2.4. *Samples from the hands of the visitor*

The samples from the visitor's dominant and non-dominant hands generated total DNA quantity ranges of 1.9–11.9 ng (av. 5.4 ng) and 1.8–6.9 ng (av. 4.1 ng), respectively (Supplementary data 1). The number of contributors detected on the hands ranged from 3 to 4 contributors (av. 3). The dog's owner was detected in 80 % of the samples (8 of 10). There was one occasion where two owners were found in the same sample associated with dog 5. An owner was detected in one of the two hands in three experiments and on both hands in the remaining two. The dog owner was always a minor or minority contributor (10 occasions, 8 of 10 samples) to the mixture and in two samples multiple dog owners were (LRs ranging from 8.2 x 10^3–4.5x10^{16}, av. 7.9 x 10^{16}).

The visitor was detected in all samples, always as a major or majority contributor (10 of 10 samples). The visitor's housemate was detected as

a minor contributor in 30 % of samples (3 of 10 samples), two of which were from the left and right hands of one visitor. Unknown, minor contributor was detected in 80 % of the hand samples (8 of 10).

3.3. Unknown donor mixture to mixture analysis

Mixture to mixture comparisons were undertaken, separate for each dog, to investigate the presence of common unknown donors possibly present on different sampling surfaces (Supplementary data 1 tab 3). Several possible common unknown donors were detected in samples relevant to all five animals.

For example, for dog 1, an apparent common unknown donor was noted on the left and right sides of the animal as well the couch arm and blanket (where the animal was known to spend time) and the visitor's outside car door handle. However, the left hand of this visitor (the only hand that had unknown DNA detected) did not result in any inclusionary LR's, above the designated cut off, during mixture-to-mixture analysis.

For the matches detected for dog 2, of note was the possible common donor (contributor 3) that was detected on both sides of the dog and both hands of the visitor, indicating that this DNA may have been picked up or deposited during the patting action. Notably, there was another possible unknown donor (contributor 2) that was detected on the right side of the dog as well as its head and bed.

For dog 3, an apparent common unknown donor (contributor 2) was detected in multiple samples associated with the visitor (such as both hands, steering wheel, phone etc), but of more interest was a possible second common unknown donor (contributor 3) that was detected on the head and back of the dog, the visitor's left hand and the outer door handle. The direction of this transfer cannot be ascertained with the present results, and it is possible that this donor was present on the dog and transferred to the hand and the car of the visitor or vice versa.

While for dog 4, a single common unknown donor was detected only on the dog and its associated surface. This was also the case for dog 5, where most samples with a single common unknown donor were associated with the dog (e.g., head, sides, back) but also the gear stick sample (contributor 3). It should be noted that while, across the five dog's samples, several LRs provided high degree of support, there were also samples that produced lower values that are likely to be reduced further if theta was incorporated into the calculation.

4. Discussion

The amount of quantifiable human DNA varied within and between different areas sampled fon the dogs, with the back providing the highest quantities. The quantity of DNA recovered, in general, was higher than that in previous studies [21,26,27], which may be attributed to differences in experimental conditions such as the duration of contact with the visitor (5 min vs 8 pats and scratches), areas sampled (head, back and sides vs head, back, skin, stomach, ears, nose/mouth) and/or collection methodologies (single swab immediately after contact vs double swabbing an hour after contact). While a study by Brower et al. [26] sampled the muzzle and teeth of a dog, after human saliva (a higher DNA containing source of biological material than touch DNA sampled in this study) had been deposited on the area and the study by Oefelein et al. [27] that collected background DNA samples without deliberate contact with a participant.

Regarding direct transfer during contact (patting), in Monkman et al. [21], DNA transfer to the dogs was observed in 35 % of the samples which was similar to the transfer rates seen in this study (35 %). Duration of contact and number of areas contacted did not appear to affect the transfer rates. For example, the visitors in this study were allowed to contact the dog on multiple areas for 5 min while in the previous study a short pat of few seconds was the type of contact tested. Several studies have shown that longer and more forceful contacts can result in increased detection [4,28], however this was not observed in the current study. The amount of pressure applied, when contacting a dog while playing with it, may also have impacted DNA deposition. Contact type could not be quantified here given visitors were allowed to interact with the dog in an unstructured manner as they felt comfortable. In one experiment, the visitor picked the dog up (dog 5) during the interaction and was one of the two visitors that had owner's DNA on both hands. While owners were asked not to pet their dog in the hour after the visit, multiple owners reported contact. In particular, three owners indicated touching their dog prior to dog sampling. Of the body areas assessed, the back was the area with the least amount of visitor's DNA, which may reflect the surface area sampled or removal of visitor's DNA by owners during the wait period. Interestingly, while no patting instructions were given, the areas contacted included the head, the back and the sides and were similar to the areas identified as being commonly touched in a pervious study [24]. If a dog is present at a scene, and based on case circumstances, these areas should be targeted first, unless other information is available. As expected, the visitor was detected in samples from their car [37,38] and homes [8,39–41]; presumably from multiple direct contacts. Another factor which was not investigated is that of the breed of the dog and the length of the hair as too few dogs of each breed and length of hair were included in this study. The impact of these variables could be considered as a focus in future studies.

Several instances of indirect transfer were noted, both from the visitor to the dog and from the dog to the visitor. Regarding the indirect transfer to the dog, the visitor's housemate, that was not part of this experiment, was detected on one of the dogs. This housemate had no direct contact with the visitor for over a week nor frequented the visitor's car during that period. However, this housemate's DNA was detected in the visitor's car likely from this person's DNA being picked up (either from the items and surfaces in the shared house and/or from the car) and transferred to the animal in a multi-step transfer event. DNA from the dog's owners was also found on items and surfaces contacted by the visitors after animal contact. Interestingly, while this was observed in relation to four of the five dogs, the owner of one dog (dog 1) was not detected in any of the indirect transfer incidents. Dog 1 was one of the two dogs with the smallest amounts of owner's DNA found on its body. It is possible that this owner was a low shedder, something that was not tested in this study, contributing to this lack of detection. Shedder status, or an individual's biological propensity towards shedding DNA [29–31], is known to impact DNA deposition and subsequent transfer detection [32,33]. In regard to the indirect transfer from the dog to the visitor (and associated items), dog owners were detected on several items in the visitors' houses. Notably, in many instances, the first contacts the visitors made in their house did not transfer detectable levels of dog owner's DNA, while later contacts (for example with the mug and kettle) did. This suggests that DNA may not be transferred with every contact and may instead be dependent on contact surface type, as well as duration and type of contact made. Generally, majority of visitor contacts were brief and only involved the fingertips, while contacts with the kettle and the mug involved the whole hand or both hands. Further, household items contacted including door handles and laptops, may have been saturated with visitor's DNA, as they generated higher DNA quantities relative to the kettle and the mug, possibly drowning relatively minor owner contributions to these samples. The concept of indirectly transferred DNA has been widely reported [5,7,34,35], including instances when the intermediary is a dog or a cat [21,22,27]. However, Oefelein et al. [27] detected very little DNA transferred from dogs to plastic cards when it was rubbed firmly against the head, back and mouth of the dog. This may be a result of the material used or differences in experimental set up (size of area sampled and type of contact) and sample processing. In the current study, indirect transfer was detected in 31 % of the samples where dog owner's DNA was detected on the visitor related surfaces and the visitor's housemate's DNA was detected on the dog and its associated items. The high incidence of multi-transfer detection may, in part, be a consequence of prolonged contacts made in this study. Notably, mixture inversions, where indirectly transferred DNA is detected as a major or majority contributor, are

rare [4,34]. In the present study, concurring with previous research [36], mixture inversions were observed in 4 % of the samples.

Finally, unknown donors were detected in most samples (71 %). Based on the mixture-to-mixture comparisons, common unknown donors were detected on the dogs, the surfaces the dogs came into contact with post-interaction, and the visitor's items representing further incidences of multi-transfer events. For example, in one instance (dog 2), an unknown donor's DNA was found on the dog and the hands of the visitor. Detection of unknown donors is common, but its origin is difficult to investigate. It is unknown if these donors originated on the visitor and their items or vice versa, but if included in the incidence of indirect transfer estimation, the number of such transfers in our studies would increase further. Further, some of the unknown contributors were exclusively found either on the dog or its contacted surfaces. This DNA may have come from multiple direct contacts (for example from household visitors) or through multi-step indirect transfer (for example DNA picked up from the outside environment and brought into the house).

5. Conclusions

This research demonstrates that dogs can easily serve as both reservoirs for human DNA and vectors for subsequent transfer, indicating potential ramifications for some crime scene investigations. This transferred DNA may reflect direct contacts made innocently (e.g. a dog owner or a guest patting or playing with the pet) or be a part of a criminal activity (e.g. contact between an intruder and the dog during a criminal act). The need to differentiate the DNA associated with these contacts introduces a layer of complexity to forensic analyses. These results underscore the importance of considering dogs, and potentially other household pets, as potential sources of human DNA relevant to an investigation.

Future research should explore DNA transfer and persistence with regards to household pets in different environments. Such projects would generate data to allow better evaluation of the DNA evidence found at scenes where animals are present, increasing the likelihood of identifying a POI and assisting forensic investigators and legal arbiters resolving cases involving pets.

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Heidi Monkman: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Roland A.H. van Oorschot:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mariya Goray:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scijus.2025.101312.

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