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Case Report

DNA profile of dog feces as evidence to solve a homicide

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ABSTRACT

Dog fecal samples were collected at the crime scene and from the shoes of the suspect to see whether they could be linked. DNA was genotyped using a 145 bp fragment containing a 60 bp hotspot region of the mitochondrial DNA (mtDNA) control region. Once the species origin was identified, sequences were aligned with the 23 canine haplotypes defined, showing that evidence and reference had 100% identity with haplotype 5. The frequency of haplotype 5 and the exclusion power of the reference population were 0.056 and 0.89, respectively. The forensic index showed that it was 20 times more likely that the evidence belonged to the reference dog than to some other unknown animal. The results support that the mtDNA hypervariable region 1 (HV1) is a good alternative for typing in trace or degraded casework samples when the STR panel fails, and demonstrate the utility of domestic animal samples to give additional information to solve human legal cases.

1. Introduction

Non-human DNA analysis in forensic science has seen growth in recent years. Applications range from investigations of crimes of humans to cruelty and poaching in animal/wildlife species, where DNA evidence from animals, plants, bacteria and viruses has been used in criminal investigations [1].

Animal Forensic Genetics is defined as "The application of relevant genetic techniques and theory to legal matters, for enforcement issues, concerning animal biological material" [2]. Domestic animal genetic evidence has become an important forensic tool for identification and individualization purposes. Interest in animal genetic evidence has recently increased [3] due to the abundance of animal evidence encountered at crime scenes [4]. Transfer of DNA from hair, saliva, blood, urine or feces can occur during the commission of a crime, from the pet of a victim to the suspect or crime scene, and from the pet of the suspect to the victim or crime scene [5].

In Argentina, the pet population is around 9 million dogs and 3 million cats, without counting stray dogs [6,7]. Because of their close relationship with people, determination of the genetic profile of pets would provide a valuable forensic tool.

Canine biological materials including hair, feces and saliva can be found when contact between dogs and humans takes place. Most of the described collection, sampling, and extraction are used in medical diagnostic applications [8,9], wildlife population [10,11] and wildlife illegal traffic studies [12]. Fecal DNA is often degraded due to environmental factors and continued active deterioration by the large numbers of bacteria present with the feces. Also, feces contain many known PCR inhibitors such as bile salts [13]. As fecal samples are not commonly received in forensic laboratories, our study sample was a challenge because the defecator DNA was extracted from cells on the surface of feces.

Mitochondrial DNA (mtDNA) markers and a standardized STR panel are used to determine the canine genetic profile. Specifically, hypervariable regions (HV) 1 and 2 in the mtDNA control region have been used to solve forensic casework [5,14–17]. Although mtDNA analysis has a lower power of discrimination than multiple nuclear STR or single nucleotide polymorphism (SNP) markers, the high copy number per cell and the uniparental inheritance make mtDNA analysis useful in certain forensic cases, particularly when the available amount of DNA is poor or degraded. In addition, a high substitution rate and a high density of polymorphisms within the non-coding mtDNA HV region allow informative sequence analysis of relatively short regions in forensic DNA analysis [18].

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The aim of the present case report was to describe how we obtained a genetic profile from a highly degraded DNA purified from dog fecal samples using an mtDNA control region locus to solve a robbery with homicide.

2. Materials and methods

2.1. Biological samples

A sample of dog feces (reference) was collected from the house where a robbery with homicide occurred. A suspect was arrested, and another sample of dog feces was collected from his shoes (evidence) in order to connect the suspect with the crime scene. Both feces samples were sent to our laboratory (IGEVET, UNLP-CONICET LA PLATA) for DNA analysis by the Office of the Prosecutor of the Province of Buenos Aires (Buenos Aires, Argentina).

2.2. DNA extraction and genotyping

DNA from feces samples was purified using two alternative methods, ZR Fecal DNA MiniPrep (Zymo Research, CA, USA) and QIAamp DNA Investigator kit (Qiagen, Germany), following the instructions of the supplier. DNA quantity and quality were measured using a NanoVue spectrophotometer (GE Healthcare, USA) and electrophoresis in 1% – 0.5 X TBE agarose gels (Fig. S1a).

In order to determine if both feces samples (reference and evidence) belonged to the same dog, 15 canine STR (AHT121, AHT130, AHTh260, AHTk211, AHTK253, FH2054, REN105L03 REN162C04, INRA21, REN169018, REN169D01, REN247M23, REN54911, REN64E19 and AMELOGENIN) were used. This STR panel belongs to the standardized and recommended list of the International Society for Animal Genetics (ISAG, http://www.isag.org.uk) for canine genetic identification.

In addition, two fragments of 800 and 145 bp of the canine mtDNA control region were analyzed. The first fragment, containing the entire HV1 region, was amplified using primers H15360 and L16106, as proposed by Himmelberger et al. [19]. The second one, containing a 60-bp highly polymorphic fragment, was amplified with primers H15575 and L15684, as published by Baute et al. [20]. This 60 bp region is a hotspot for SNP within the canine mtDNA, allowing the discrimination of most of the described haplotypes [20].

Both fragments were amplified in a total volume of 25 μl of the following reaction: 1X buffer, 0.25 mM $MgCl_2$, 0.8 mM dNTPs, 0.5 X enhancer (Inbio Highway), 0.04 U/μl T-Holmes DNA polymerase (Inbio Highway) and 5 pmol of each primer. The PCR program included an initial denaturation step for 2 min at 94 °C, followed by 35 cycles of 15 s at 94 °C, 30 s at 51 °C (800 bp fragment) or 56 °C (145 bp fragment), 1 min at 72 °C, and a final extension for 10 min at 72 °C.

PCR products were purified with polyethylene glycol (PEG) 8000 and sequenced in an automatic DNA MegaBACE 1000 sequencer (GE Healthcare) using DYEnamic ET Terminator Kit (GE Healthcare). Raw sequences were edited with Sequence Analyzer (GE Healthcare). The identity of the obtained sequences was determined using the Basic Local Alignment Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi). DNA sequences were aligned to the reference dog sequence (U96639.2) using DNAMAN version 4.15 (Lynnon BioSoft, Quebec, Canada) and UGENE software [21]. Polymorphic sites were defined by comparison. Haplotype variants were identified based on the 23 canine haplotypes defined by Baute et al. [15].

2.3. Statistical analysis

In order to establish the haplotype frequency database, all canine mtDNA control regions reported in the Genbank database (http://www.ncbi.nlm.nih.gov/genbank/) were blasted using the 60 bp region of the reference sequence and the result was downloaded as an html file. The recovered sequences were filtered with PhyloclassTalk software (http://biosmalltalk.blogspot.com.ar/2013/02/phyloclasstalk-preview.html) using the following parameters: Identity = 98%; hit definition = *Canis lupus familiaris*|*Canis familiaris;* alignment length = 57–59; breed group = main dog breeds from Argentina (Federación Cinológica Argentina, FCA, http://www.fca2000.org.ar/; Table S1). The filtered DNA sequences were exported to the Arlequin software [22] for haplotype frequency estimation. The exclusion power of the 60 bp canine sequence located in the HV1 region was estimated as:$EP = 1 - \sum_{i=1}^{23} x_i{}^2$where x_i is the frequency of the ith haplotype and $\sum x_i^2$ is the random match probability [19]. The forensic index ("likelihood ratio", LR) was estimated as: $LR = \frac{1}{xi}$ where x_i is the expected frequency of the haplotype in the population [23].

3. Results and discussion

The quantity and quality of the DNA extracted with both kits were similar (Table S2 and Fig. S1a). The amount of DNA obtained was within the range reported by Lindquist et al. [13]. Genotyping of the STR panel showed no positive results (Fig. S1b), probably due

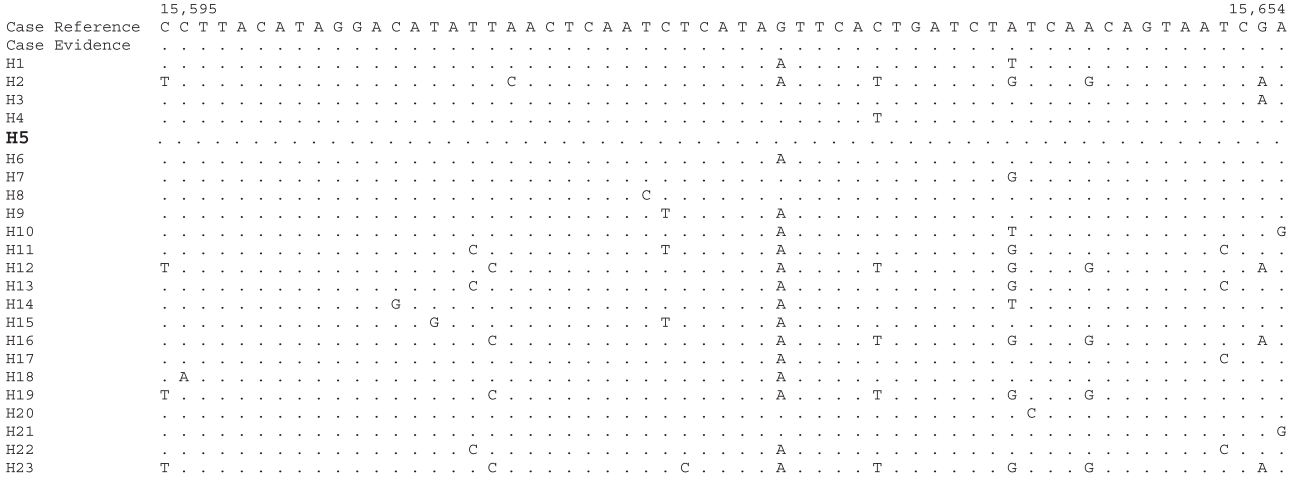

Fig. 1. Alignment of the highly polymorphic fragment of the canine mitochondrial DNA HV1 region obtained from the casework evidence and reference samples with the previously reported haplotypes (H1–H23; Baute et al., 2008).

Table 1
Exclusion power (EP) and likelihood ratio (LR) values estimated for the "breed filtered", the "non-breed filtered" and the "Baute et al., 2008" reference population database. N = number of animals, n_h = number of haplotypes.

	All breeds	Argentine breeds	Baute et al. (2008)
N	2930	483	242
n_h	23	17	23
EP	0.91	0,89	0.82
LR	11.76	20	14.26

to the low quality of the obtained canine DNA. Furthermore, most of the purified DNA could correspond to microbial sources; thus, the method used to quantify the DNA extracted was not species-specific. Concerning HV1 fragments, only the 145 bp fragment could be amplified and sequenced (Fig. S1c). These results agree with those previously reported by Baute et al. [20] who suggested that this region could be used successfully for typing trace or degraded casework samples. By contrast, amplification of larger fragments failed in this condition [20].

Alignment of the 60 bp sequence evidenced that the DNA sequences obtained from feces samples matched with those from the species *Canis lupus familiaris* (domestic dog), thus excluding other species sources and confirming the robustness and specificity of the assay to exclude non-contributing dogs [20]. Comparison with the 23 canine haplotypes defined, based on the 60 bp fragment, showed that both evidence and reference had 100% identity with the canine mtDNA control region haplotype 5 (Fig. 1).

On the basis of all previously reported sequences, the reference population resulted in a recovery of 3568 dog mtDNA sequences. These sequences were filtered as mentioned above using the PhyloclassTalk software. Then, the new database formed by 2930 sequences was filtered again using the breed dictionary module of the PhyloclassTalk software, considering the main canine breeds raised in Argentina. The final database contained 483 DNA sequences corresponding to 17 out of the 23 canine haplotypes, and was used as population database ("breed filtered") to estimate the gene frequencies (Table S3). Furthermore, the gene frequencies reported by Baute et al. [20] and the entire GenBank data base ("non-breed filtered") were used as alternative reference populations. As shown in Table S3, the gene frequency of haplotype 5 was 0.056 in the "breed filtered" reference population, 0.086 in the "non-breed filtered" ones, and 0.070 in Bautés database.

Using the estimated gene frequencies, the exclusion power of the 60 bp mtDNA control region fragment varied from 0.8 to 0.91, depending on the database used. These values agree with previous reported data (EP = 0.89) [19]. Regarding the weight of the evidence in the analyzed casework of robbery with homicide, the resulting LR varied from 11.76 to 20, also depending on the database used (Table 1). Thus, the evidence showed that it was 20 times more likely that the reference feces sample belonged to the same individual found at the crime scene than to some other unknown animal.

4. Conclusions

DNA purified from feces samples has been extensively used in human medicine and wildlife population studies. In criminal investigation, it is a challenge due to the low number of cells, the degraded DNA and PCR inhibition. However, in the present study, we demonstrate in a real forensic casework the usefulness of the analysis of a short fragment of the canine mtDNA control region with the PhyloclassTalk software to give additional evidence to connect a suspect with a victim and a crime scene, supporting the prosecutoŕs accusation and in this way contributing to solving a case of robbery with homicide. Furthermore, the present results support that the 60 bp mtDNA control hotspot region is a good alternative for typing in trace or degraded casework samples where it is difficult to obtain a complete mitochondrial or autosome genetic profile. Finally, we demonstrate the utility of domestic animal samples, such as feces, to assess additional evidence to solve human legal cases.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.legalmed.2016.08.002.

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