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The first criminal case in Estonia with dog's DNA data admitted as evidence

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Abstract

Dog's hairs and possible saliva stains were recovered from the coat of a dead woman, possibly attacked by a dog(s). The reference hairs and saliva samples were collected from different dogs living in the neighbourhood. Mitochondrial and nuclear DNA analysis were used to analyze the crime scene and reference samples. Despite many samples analyzed and a lot of DNA data obtained, it was not possible to determine which dog's hair and saliva were on the murdered woman's coat, as all reference dogs/samples had been excluded.

Keywords: Dog; Forensic DNA analysis; Nuclear markers; Mitochondrial DNA

1. Introduction

Nuclear and mitochondrial DNA of human origin have proven their importance in criminal investigation for human identification purposes, as well as for establishing a link between crime scenes and certain persons. Currently, forensic DNA analysis is being extended to cover the usage of DNA from other species involved in crime. This extension includes the usage of DNA from microorganisms (viruses, bacteria, etc.) and macro-organisms (dogs, cats, etc.) and covers both the species level and the individual level. Our study describes the first attempt in Estonia to use dog's DNA analysis (nuclear and mitochondrial DNA) in a criminal investigation.

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2. Case story

A body of a young woman was found in the field near a small village. The forensic examination suggested that the woman had been attacked by a dog (or dogs). Dog hairs and possible saliva stains were recovered from the coat of the woman. The reference hairs and saliva samples were collected from six different dogs living in the neighbourhood. As two dogs had gone missing after the accident, some hairs from the kennel of the farm with missing dogs were collected. Five months after the accident, two vagabond dogs were found not very far from the district of the woman's death and were executed by police. Those samples were also submitted for DNA analysis.

3. Material and methods

3.1. Samples for DNA analysis

Hair samples consisted of the hair with the sheath material from the coat of the woman (sample 1), hairs from the kennel place (samples 2 and 3) and hairs from the kennel (samples 4, 5 and 6). Saliva samples were (I) from the dogs of the first farm (the farm of the missing dogs): the black curly mongrel (sample 7) and the reddish sleek mongrel (sample 8); (II) from the dogs of the second farm: the Rottweiler (sample 9); (III) from the dogs of the third farm: the black mongrel (sample 10), the German shepherds (samples 11 and 12); and (IV) two samples from the woman's coat (samples 13 and 14).

Soft tissue (brain) samples were from the slaughtered light beige dog (similar to the Caucasian sheep dog) (sample 15) and from the killed greyish black dog (similar to the German shepherd) (sample 16).

3.2. DNA extraction

The organic DNA extraction method was used for the DNA extraction from hair samples. The Chelex-100 method was used for the DNA extraction from the saliva and soft tissue samples.

3.3. Mitochondrial (mt) DNA analysis

The 148-bp segment of the hypervariable region 1 (HV1) was amplified using the primers D5 and D8 [1]. In the cases where PCR product was not detectable by agarose gel electrophoresis, seminested PCR was carried out, using the primer D8 and the primer D7 [1]. The size of the product of the second PCR amplification was reduced to 125 bp. The primer D8 was designed to carry M13–21 sequence at the 5′-end to enable direct sequencing of PCR products. The PCR conditions were in accordance with the literature [1]. PCR products were fractionated by agarose gel electrophoresis. DNA was purified using PCR Purification Resin (Promega, USA). The purified PCR products allowed for direct DNA sequencing, using DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech, USA). The sequence reactions were analyzed an ABI PRISM

310 Genetic Analyzer (Applied Biosystems, USA). Comparisons were performed using the Sequencher program (GeneCodes, USA), taking into account the reference sequence from the NCBI database (accession no. AF008147, *Canis familiaris* isolate D26a mitochondrial control region I, D-loop sequence).

3.4. Nuclear DNA analysis

The StockMark Kit for Dog Parentage Verification and Identification (Applied Biosystems) was used for nuclear DNA amplification. PCR reactions were performed using manufacturer's suggested PCR conditions. The PCR products were analyzed on an ABI PRISM 310, using GS500 ROX size standard.

4. Results and discussion

4.1. Mitochondrial DNA analysis

Out of 16 samples, only one hair sample (sample 2) did not yield successful PCR amplification. Two hair samples (samples 1 and 3) gave a result after seminested PCR amplification which provided shorter PCR products. All PCR products (except for sample 2) were sequenced. Most of the DNA sequences were identical (Table 1). Altogether, seven polymorphic positions were found, two of them (positions 46 and 53) differed only

Table 1
Summary of results of mitochondrial DNA analysis

Sample	Polymorphic position						
	23	31	34	35	46	53	59
Reference sample	G	T	C	T	G	C	A
1	*	*	*	*	A	G	*
2	nn.	nn.	nn.	nn.	nn.	nn.	nn.
3	*	*	*	*	A	G	*
4	*	*	*	*	A	G	*
5	*	*	*	*	A	G	*
6	*	*	*	*	A	G	*
7	*	*	*	*	A	G	*
8	*	*	*	*	A	G	*
9	*	*	*	*	A	G	*
10	*	*	*	*	A	G	T
11	*	C	T	A	A	G	T
12	A	*	A	*	A	G	*
13	*	*	*	*	A	G	T
14	*	*	*	*	A	G	*
15	*	*	*	*	A	G	*
16	*	*	*	*	A	G	T

The numbering of polymorphic positions starts at the 3′ -end of the primer D8. Reference sample is database sample as mentioned in the text.

from the reference sequence without actual polymorphisms in between the analyzed sequences. Surprisingly, the difference between two saliva samples from the woman's coat was established at position 59. At the same time, the nuclear DNA analysis of those samples revealed the same results at all analyzed loci. A number of studies have shown heteroplasmy and mosaicism in human mtDNA [2], thus, it would not necessarily be correct to conclude from these results that the two saliva samples on the woman's coat have different origins, if they differ in only a single nucleotide position in the mtDNA. Based on the mtDNA analysis, it was possible only to exclude two dogs as potential donors of saliva and hair samples.

4.2. Nuclear DNA analysis

The nuclear DNA analysis of saliva and tissue samples provided complete DNA profiles (Table 2). Only one hair sample (taken from the kennel) gave a complete DNA profile. The data obtained from other hair samples were incomplete, presumably due to nuclear DNA degradation. Based on the nuclear DNA analysis (even if the profiles were incomplete), it was possible to conclude that DNA found in the evidence saliva samples did not match DNA contained in the reference hairs, saliva and tissue samples submitted for DNA typing. Consequently, these evidence samples could not have originated from these dogs. The hair collected from the coat differed from saliva samples from the same coat, but the concordance with DNA data obtained from such a hair and other samples was not established, thus, the origin of the hair is still unknown. The hairs taken from the kennel did not originate from the dogs of that farm whose saliva samples were analyzed, but might originate from their close relatives.

Table 2
Summary of results of nuclear DNA analysis

Sample	DNA loci									
	PEZ 1	FHC 2054	FHC 2010	PEZ 5	PEZ 20	PEZ 12	PEZ 3	PEZ 6	PEZ 8	FHC 2079
1	110	163	223	110, 114	175	–	105, 117	176	–	277
2	110	–	–	120	–	–	–	–	–	–
3	110	–	–	120	–	–	–	–	–	–
4	110, 118	158,163	223	110, 114	175	268, 276	117, 120	176, 180	228, 236	277, 289
5	114, 118	–	–	114	175	–	–	–	–	–
6	118	163	–	110, 114	–	268, 276	120	176	–	–
7	110, 118	163, 179	223	110, 114	175, 179	276, 295	120	176, 184	228, 236	277, 289
8	110, 114	175, 179	223, 235	106, 110	175, 183	268, 276	120	184, 188	228, 236	269, 289
9	106, 118	154, 175	223	102, 106	175, 179	272, 295	120, 129	180, 192	224, 232	285, 289
10	118, 122	149, 158	223, 235	102, 110	175, 179	272, 276	129, 135	168, 176	236, 240	269, 273
11	118	149, 167	223, 235	102	175	268	129	172, 188	228, 236	269, 273
12	118	163	223, 227	102	175	268	129	176, 188	224	273
13	118	149, 179	223, 227	102, 114	175	268, 276	117, 120	184	236	277
14	118	149, 179	223, 227	102, 114	175	268, 276	117, 120	184	236	277
15	118	163, 171	231	102, 110	187	264, 268	–	176, 180	236	269
16	114, 118	149	223	102	175	272, 302	–	168, 180	240, 244	298

5. Conclusions

(1) The mtDNA analysis of 16 different samples revealed only four different haplotypes. Due to a very low exclusion capacity, it was possible to exclude only two dogs.

(2) The nuclear DNA analysis of 16 different samples revealed 14 different DNA profiles (even though some of them were incomplete DNA profiles). Two DNA profiles obtained from saliva samples from the woman's coat were identical in all the ten loci analyzed, so were two DNA profiles from two hair samples, in which only two alleles were detectable. Despite many samples analyzed it was not possible to determine whose dog's hair and saliva were on the murdered woman's coat as all reference dogs/samples had been excluded.

References

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