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Importance of canine identification in the Hungarian forensic practice

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Abstract

Hungary has an old long cynophilic and cynologic tradition, and a significantly high canine population. However, during the last years, several (even lethal) attacks to man were observed. In some cases, the human remains on the dog's body or stomach content can prove the animal as perpetrator. Lacking this evidence, the availability of certain canine-specific polymorphic STR loci is a significant tool for identification of canine individuals by their remains in the victim's clothes or the environment of the attack to solve such cases.Ten canine-specific STR loci were analyzed by fluorescently labeled multiplex PCR. The automated data collection was performed and compared applying fluorescent ladder. The forensic practice requires the availability of sequenced allelic ladders—which are constructed—and data concerning the frequency of the alleles in the local canine population (population studies are examined continuously in mixed and purebred populations).

Keywords: Canine; Identification; Forensic; Microsatellites; Multiplex STR profiling

1. Introduction

Several (often lethal) attacks to humans were caused by dogs in Hungary during the last years. In some cases (Case 1), the human remains on the dog or in the dog's stomach can prove the animal as perpetrator. When there is a lack of these evidences (Case 2), the availability of certain canine-specific polymorphic STR loci is a significant tool to identify the canine individuals by the remains in the victim's clothes or the environment of the attack, to solve such cases.

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Due to the increasing number of cases a new project started with the main aim to establish a canine identification system in the Hungarian forensic practice with the application of commercially available canine specific STR markers in different types of samples e.g. saliva, hair etc. [1,2]. According to recent population studies the degree of polymorphism of the 10 microsatellite markers (PEZ1, FHC2054, FHC2010, PEZ5, PEZ20, PEZ12, PEZ3, PEZ6, PEZ8, and FHC2079) [3] seems to be sufficient for canine identification. These studies are complemented by the construction of allelic ladders controlled by sequencing and by the availability of data concerning the allele frequencies in the local canine populations. This information comprises sufficient tools for canine identification in the routine Hungarian forensic practice.

2. Case reports

2.1. Case 1

November 27, 2000, Budapest. Two dogs of the American Staffordshire terrier or American pit bull terrier breeds (or a cross of the two breeds) passed through the fences into a garden and attacked an old lady. Thank to the rapid police actions, the stomach content, as well as bloodstained hair samples from the dogs' muzzle and hindquarters were collected. The examinations revealed tissue remains of human origin in the stomach content of both dogs. The genetic profile of the tissues and the bloodstains on the dog hairs was found to be identical with the victim's profile.

2.2. Case 2

January 17, 2001, Budapest. According to the testimony of the stepmother, the Alaskan malamute dog of the family caused the death of both of her 1-month-old twins. Due to the seriousness of the case, the police demanded the exposition and identification of canine biological remains on the twins' clothes. Additionally, blood and hair samples were collected from the dog for comparison. The experts' examinations revealed human bloodstained remains of canine origin, very possibly saliva. The canine genetic profile obtained was identical to the dog's one and the human genetic profiles obtained from the blood stains originated from the twins.

3. Materials and methods

Body tissue parts were collected from the stomach content of the dogs. The bloodstains on the hairs were removed and collected.

Several textile pieces were cut from and collected from both twins' clothes. Blood samples from the victim (Case 1) and the malamute (Case 2) were used for comparison.

From the body tissue cells, the blood on the hair and the blood samples DNA was extracted. From the stains on the twin's clothes, the pellets underwent differential lysis to obtain epithelial and blood cells.

Table 1
Detected sizes of amplified fragments at canine loci in case of crime samples and suspected dogs

Sample fraction$_1$		PEZ1	PEZ1	FHC 2054	FHC 2054	FHC 2010	FHC 2010	PEZ5	PEZ5	PEZ20	PEZ20
Stains$_{1-7}$	Mean$_{nt}$	115.84		150.41	154.49	220.19		106.73		171.21	175.12
	S.D.$_{nt}$	0.01		0.04	0.03	0.04		0.04		0.05	0.12
Dog		115.84		150.45	154.50	220.16		106.76		171.17	175.22
		PEZ12	PEZ12	PEZ3	PEZ3	PEZ6	PEZ6	PEZ8	PEZ8	FHC2079	FHC2079
Stains$_{1-7}$	Mean$_{nt}$	265.63	298.38	123.79		175.65		216.22		274.28	
	S.D.$_{nt}$	0.20	0.19	0.07		0.05		0.08		0.11	
Dog		265.63	298.47	123.79		175.63		216.19		274.34	

nt = Nucleotide.

The DNA of human origin was coamplified by use of a commercially available PCR amplification kit (Profiler Plus, Applied Biosystems, Foster City, CA). The suspect canine DNA samples were coamplified by decaplex PCR reaction with the use of the StockMark Kit Canine I Ver.3 (Applied Biosystems). The fluorescence-based automatised detection of the PCR products was performed by ABI PRISM 310 Genetic Analyzer by the application of CXR 60-400 (Promega, Madison, WI) fluorescence internal standard.

The fragments of the allelic ladders of the canine-specific loci were amplified by the use of monoplex PCR. The separation and purification of the PCR products was done after acrylamide gel electrophoresis following standard procedures, and the forward and reverse sequencing was performed as well (by the application of ABI PRISM BigDye Terminator Cycle Sequencing Kit and ABI PRISM 377 DNA Sequencer). The determination of the fragment sizes of the constructed allelic ladder was carried out by ABI PRISM 310 Genetic Analyzer and CXR 60-400 as well as GeneScan 500 ROX and GeneScan 400HD ROX (Applied Biosystems) internal sizing standards.

4. Results and discussion

In the first case, in spite the fact that the digestive functions of the dog are relatively fast, thanks to the rapid actions of the police, the demonstration of human tissue remains in both dogs' stomach contents and their identification was successful—PM: 7.06×10^{13}.

In the second case, the PCR amplification from the mixed human–canine samples (total 1–3 ng/μl DNA) was successful (Table 1). According to the sequenced fragments, the construction of a nomenclature of alleles based on the repeat-number is possible. The determination of the fragment length depends on the internal standard applied, the least S.D. values were calculated by CXR 60-400 internal standard at PEZ20 locus. The PM values of the samples' genetic profiles were calculated by the observed frequency of the fragments in our population sample ($N = 484$) and they were PM = 7.14×10^{13} in case of the Alaskan malamute. Due to the effect of inbreeding in local purebred subpopulations [4], extended studies including a larger number of individuals per breed are required.

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