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CASE REPORT

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Canine microsatellite polymorphisms as the resolution of an illegal animal death case in a Hungarian Zoological Gardens

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Abstract Several animal carcasses were found in the paddocks of a Hungarian County Zoo during 1 week. The 14 animals killed were thought to be the victims of a dogfight training. The primary suspect was the security guard of the Zoo with his guard dogs. DNA tests were carried out on hairs and bloodstains and 10 canine-specific STR loci were analysed by fluorescently labelled multiplex PCR using the ABI PRISM 310 Genetic Analyzer. The results confirmed that the killer was a single animal and all of the guard dogs were excluded.

Keywords Canine · Animal identification · Hair · Microsatellites · Multiplex STR profiling

Introduction

The recognition and importance of animal rights is emerging world-wide and apart from the ethical aspects, animal fighting could have consequences for people and property which could be manifested as crime. In some cases not only humans but even canine individuals could be involved as a putative perpetrator or accessory to a crime [1, 2]. The forensic DNA typing of human biological trace evidence by STR (short tandem repeat) examination employing commercially available multiplex PCR methodology is becoming the prevalent technology. The genetic testing of wild animals [3] and the breeding aspects of accurate determination of relatedness of animals [4] has resulted in several polymorphic loci which can also be applied in forensic casework [5, 6, 7]. The specialities of forensic casework analyses are often analogous to examination of anomalous biological evidence material such as hairs. In contrast to humans, hairs usually have a shorter anagen phase and a longer telogen phase in most other mammals [8, 9]. The effect of the natural and seasonal shedding of the animal coat manifests a certain variability in the cellular content at the hair root in the case of a traumatic event. The occasional limitation of DNA quantity of a single hair must be considered as having an influence on the varying success rate of STR and even mtDNA analyses [10].

The goal of this work was to test commercially available canine-specific STR markers from limited quantities of biological samples such as single hairs. The 10 microsatellite markers used (PEZ1, FHC2054, FHC2010, PEZ5, PEZ20, PEZ12, PEZ3, PEZ6, PEZ8 and FHC2079) [11] appear to be efficient for the resolution of such types of cases. Due to the lack of forensically validated allelic ladders precision sizing is a suitable method of measuring the size of amplified fragments. Due to the genetically homogenous nature of inbred pedigree dogs the exact forensic application of these loci (eg. inclusion) requires population studies of local canine populations [4, 12].

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Case report

Several not severely damaged carcasses of wallabies (*Macropus rufogriseus* and *Macropus parma*) and maras *(Dolichotis patagonum)* were found in their paddocks in the Baranya County Zoo. A similar event was repeated with maras *(Dolichotis patagonum)* and Cameroon goats *(Capra hircus nanus)* 2 days later. On searching the crime scene a previously non-existent, clearly man-made hole in the fence was discovered. There were signs of fighting at several places on the ground. Animal hairs and a minute amount of blood were discovered as trace materials at the crime scene.

According to the findings of the post-mortem examination, the cause of death of the animals was suffocation. The veterinary autopsy revealed typical wounds made by the teeth of a carnivore but no signs that the animals were killed for feeding.

Due to the high wire fences and that the hole was made in the fence of one paddock only, the killer animal could only have entered the areas with human assistance so that after passing through

the hole and killing the animals it must have been carried over the wire fence into the neighbouring paddock in order to attack the other animals. Considering the natural aggressive behaviour of the victim animals (eg. wallaby) and the way of entering, the police investigations indicated a form of dogfight training.

The animals primarily suspected were the six German shepherd dogs which protected the Zoo with the security guard. The hairs from the crime scene were examined macroscopically and microscopically. The majority of hairs belonged to the victims, but some resembled the coat of German shepherd dogs. The microscopic morphological examination revealed a high degree of similarity with the unidentified dog hairs to two of the suspected dogs. Further DNA investigations were necessary to make a conclusive identification of the perpetrator.

Materials and methods

A total of six hairs from the manipulated gap of fence, two hairs and a small amount of smeared bloodstain from the top of bordering wire fence of the wallaby paddock and a minute amount of floss from the ground of the goat paddock were collected at the crime scene. The control samples for comparison were few plucked hairs from the corpses and from the suspected dogs. The gelatin-embedded hairs were selected for further DNA analysis by microscopic examination. The 5–8 mm-long root end of the hairs was cut and rinsed in sterile deionised water. All of the hair roots as potential DNA sources and the bloodstain were extracted by proteinase K digestion and phenol extraction followed by concentration in Centricon-30 (Amicon, Mass.) spin dialysis tubes. The highly concentrated DNA (approx. 10 µl final volume) was co-amplified by decaplex-PCR using the reagents provided in the StockMark Kit Canine I Ver.3 (PE AgGen, Foster City, Calif.). PCR amplification was carried out for 35 cycles according to the manufacturer's instructions. Capillary electrophoresis and fluorescence-based automated data collection were performed on an ABI PRISM 310 Genetic Analyzer applying the fluorescent ladder CXR 60-400 (Promega, Madison, Wis.) as the internal size standard.

Results and discussion

By the comparative microscopic examination, all the hairs found surrounding the manipulated gap in the fence were proven to be wallaby hairs. The hairs ($Hair_I$, $Hair_{II}$) from the top of the bordering fence and the floss ($Hairs_{III}$) from the goat paddock were identified as coming from a German shepherd dog. The hairs and the bloodstain ($Blood_{IV}$)

Table 1 Detected sizes of amplified fragments at PEZ1, FHC2054, FHC2010, PEZ5 and PEZ20 loci in case of crime scene samples and suspected dogs. The allele designation obtained by measuring standard deviations *(SD)* suggests a single individual as perpetrator (*nt* nucleotide)

Sample	PEZ1	PEZ1	FHC2054	FHC2054	FHC2010	FHC2010	PEZ5	PEZ5	PEZ20	PEZ20
$Hair_I$	107.68	111.77	142.50	–	224.40	232.55	107.00	–	175.59	179.53
$Hair_{II}$	107.58	111.62	142.34	–	224.38	232.49	107.58	–	175.49	179.41
$Hairs_{III}$	107.57	111.62	142.34	–	224.38	232.45	106.88	–	175.40	179.29
$Blood_{IV}$	107.68	111.77	142.40	–	224.40	232.50	106.89	–	175.39	179.31
$Mean_{nt}$	107.63	111.70	142.40	–	224.39	232.50	107.09	–	175.47	179.39
SD_{nt}	0.06	0.09	0.08	–	0.01	0.04	0.33	–	0.09	0.11
Dog 1	115.95	120.00	150.58	154.58	232.82	–	98.66	–	171.91	–
Dog 2	116.06	–	146.42	158.50	220.33	232.44	98.79	–	171.38	–
Dog 3	116.19	–	150.49	158.61	220.36	232.59	98.56	–	171.72	–
Dog 4	116.25	–	146.61	–	233.03	–	98.54	–	172.21	–
Dog 5	116.01	–	162.70	–	232.48	–	98.42	–	171.39	–
Dog 6	116.38	–	146.57	158.58	220.41	232.52	98.76	–	171.93	–

Table 2 Detected sizes of amplified fragments at the PEZ12, PEZ3, PEZ6, PEZ8 FHC2079 loci in the case of the crime scene samples and suspected dogs. The allele designation obtained by measuring standard deviations *(SD)* suggests a single individual as the perpetrator (*nt* nucleotide)

Sample	PEZ12	PEZ12	PEZ3	PEZ3	PEZ6	PEZ6	PEZ8	PEZ8	FHC2079	FHC2079
$Hair_I$	270.29	–	117.71	–	172.11	176.98	221.75	241.12	274.38	282.36
$Hair_{II}$	270.40	–	117.66	–	172.17	177.03	221.78	241.15	274.36	282.47
$Hairs_{III}$	270.14	–	117.66	–	172.21	177.05	221.66	241.07	274.23	282.33
$Blood_{IV}$	270.26	–	117.71	–	172.16	177.00	221.63	241.03	274.38	282.49
$Mean_{nt}$	270.27	–	117.69	–	172.16	177.02	221.71	241.09	274.34	282.41
SD_{nt}	0.11	–	0.03	–	0.04	0.03	0.07	0.05	0.07	0.08
Dog 1	266.61	–	120.58	123.70	168.40	178.83	222.04	–	270.68	–
Dog 2	265.75	–	123.93	126.97	184.73	–	221.66	225.65	265.75	–
Dog 3	266.35	–	127.03	–	174.98	178.83	217.57	221.70	266.35	270.36
Dog 4	266.57	–	123.92	126.92	172.21	184.66	225.88	233.03	266.57	270.40
Dog 5	266.27	–	123.92	–	182.75	196.36	221.66	232.85	270.50	–
Dog 6	266.57	–	123.82	126.96	181.07	184.88	221.90	–	266.57	270.40

were the materials submitted for further DNA analysis. The multiplex PCR amplification of extracted and highly concentrated DNA was successful in all samples and resulted in well-resolved fragments from each of the evidential materials. Computerised allele sizing was performed by applying the CXR 60-400 as the internal standard and the achieved sizing precision of ≤ 0.33 nt standard deviation allowed a ± 0.5 nt allele size window to be set for genotyping (Tables 1 and 2).

The STR analysis revealed only one genetic profile from each unknown sample of hair and bloodstain. The morphological examination of the hairs suggested that a German shepherd dog was the killer animal, but from the DNA analysis additional information to support this suspicion could not be obtained. The genetic profiles of the suspected dogs were different from the crime scene samples, resulting in exclusion of the guard dogs (Table 1 and 2). It is possible that somatic mutations may occur in hair root tissues, but the clear profiles observed and the balanced heterozygous alleles, suggest that only a single canine individual was the perpetrator in all the cases. The high similarity of the genetic profiles, especially at the PEZ5, PEZ20 and PEZ12 loci in the case of the suspected animal, could support the hypothesis of a close genetic relationship. The profile of the unknown individual includes alleles of different lengths and high heterozygosity, which support the assumption of an unrelated animal. Considering the close genetic relationship in inbred canine populations, the possibility of the presence of more than one perpetrator animal should also be considered. The statistical calculation of the probability of the presence of more than one unidentified individual requires data of allele frequencies on the loci examined and population studies on the Hungarian dog populations are currently in progress. Based on the known facts of the investigation and the forensic examinations, the police have not rejected the theory of dogfight training.

References

1. Wiegand P, Schmidt V, Kleiber M (1999) German shepherd dog is suspected of sexually abusing a child. Int J Legal Med 112:324–325
2. Schneider PM, Seo Y, Rittner C (1999) Forensic mtDNA hair analysis excludes a dog from having caused a traffic accident. Int J Legal Med 112:315–316
3. Menotti-Raymond MA, O'Brien SJ (1995) Evolutionary conservation of ten microsatellites in four species of Felidae. J Hered 86:319–322
4. Zajc I, Mellersh CS, Sampson J (1997) Variability of canine microsatellites within and between different dog breeds. Mamm Genome 8:182–185
5. Menotti-Raymond MA, David VA, Stephens JC, Lyons LA, O'Brien SJ (1997) Genetic individualization of domestic cats using feline STR loci for forensic applications. J Forensic Sci 42: 1039–1051
6. Müller S, Flekna G, Müller M, Brem G (1999) Use of canine microsatellite polymorphisms in forensic examinations. J Hered 90:55–56
7. Shutler GG, Gagnon P, Verret G, Kalyn H, Korkosh S, Johnston E, Halverson J (1999) Removal of a PCR inhibitor and resolution of DNA STR types in mixed human-canine stains from a five year old case. J Forensic Sci 44:623–626
8. Ackerman AB, DeViragh PA, Chongchitnant N (1993) Neoplasms with follicular differentiation Ch.4, anatomic, histologic and biologic aspects of hair follicles and hairs. Lea and Febiger, Philadelphia
9. Linch CA, Smith SL, Prahlow JA (1998) Evaluation of the human hair root for DNA typing subsequent to microscopic comparison. J Forensic Sci 43:305–314
10. Pfeiffer H, Hühne J, Ortmann C, Waterkamp K, Brinkmann B (1999) Mitochondrial DNA typing from human axillary, pubic and head hair shafts – success rates and sequence comparisons. Int J Legal Med 112:287–290
11. Neff MW, Broman KW, Mellersh CS, Ray K, Acland GM, Aguirre GD, Ziegle JS, Ostrander EA, Rine J (1999) A second-generation genetic linkage map of the domestic dog, Canis familiaris. Genetics 151:803–820
12. Fredholm M, Winters AK (1996) Efficient resolution of parentage in dogs by amplification of microsatellites. Anim Genet 27: 19–23