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ORIGINAL ARTICLE

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Canine-specific STR typing of saliva traces on dog bite wounds

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Abstract Forensic investigations in dog attacks usually involve the examination of bite marks and toothprints, the dog's stomach and pathological methods. For identification of the offending dog we evaluated canine STR typing of saliva traces on dog bite marks. The specificity of 15 canine-specific STRs was tested on human-canine DNA mixtures prior to an applied study in which 52 cases of dog bites were investigated. The first-aid wound bandages as well as swab samples from the surrounding area of the wound were used for DNA analyses. Generally, it was possible to obtain a canine-specific STR profile from the dog's saliva left on the wound area, even when high background of human DNA was present (blood). Interestingly, we found canine STR typing to be more successful when the bandages and swabs showed high amounts of human blood, i.e. when the dog bite was severe. Canine saliva was then sometimes visible as white-coloured secretion on the human blood surface. Less severe bite cases, which did not result in bleeding wounds, showed less success in obtaining useful STR results, probably due to the fact that the surface of the wounds may have been treated before the victims consulted medical aid which therefore removed the canine cells.

Keywords Canine STRs · STR multiplex · Saliva stain · Dog attacks · Forensic science

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Introduction

Domestic dogs are increasingly involved in forensically relevant incidents, such as accidents or dog attacks, as they are one of the most favourite pets in households. Reported dog populations amount to 25 million in the United States [1, 2], 4.7 million in Victoria (Australia) [3], 4.8 million in Germany and 550,000 in Austria (http://www.starkehunde.com/newsroom/Onlinebuch.pdf). In Hong Kong 200 dog bites are recorded every month and in Germany 30,000 dog bite injuries are registered every year [2]. Estimates of dog bite incidences in the United States vary from 3.5 to 4.7 million bites per year [1]. In Austria 2,845 dog attacks were registered in the year 2003 (http://www.bmgf.gv.at). The highest injury rate is reported for children between 5 and 9 years old, while adults are less affected [1, 3, 4, 5, 6, 7, 8, 9, 10, 11]. Children are more likely to be bitten on the face, neck and head and adults bitten on the extremities [1, 3, 5, 6, 10] due to height differences. The number of deaths resulting from dog attacks in the U.S. was reported to be 238 between 1979 and 1998 [2]; in Germany 55 fatal dog bite injuries were registered from 1968 to 2002 (http://www.starkehunde.com/newsroom/Onlinebuch.pdf). The death rates from dog bites in Australia and the United States range between 0.004 and 0.07 per 100,000 inhabitants [3].

Although the majority of victims were bitten by their own dogs, bites inflicted by free-running and/or stray dogs are quite common [1, 3, 5, 6, 7, 9, 10]. The German Shepherd, Rottweiler and Pit bulls, as well as their crossbreeds, head the statistics of dog breeds involved in bite injuries [1, 3, 5, 7, 9, 12].

Individual dog bite injuries were reported in case studies in which DNA profiling of hair, blood or saliva of either the biting dog, the victim or both was successful. This complements other methods for the investigation of fatal dog attacks, such as the examination of bite marks and the dog's mouth and stomach as well as pathological methods [2, 13, 14, 15, 16].

In order to investigate canine-specific STRs on dog bite marks in a more systematic approach, we examined 52

dog attack cases, in which the victims were examined at the Department of Trauma Surgery, of the University Hospital Innsbruck. We used the swabs and wound bandages (Fig. 1) for extraction and analysis of the canine-specific DNA. The following assumptions have been taken into consideration:

1. The analysis of canine DNA from swabs and bandages directly from the wound and its surrounding area will be of stronger evidence concerning the identity of the perpetrator compared with other means of evidence such as hair samples or blood and saliva stains found on the clothes of the victim.
2. The remnants of canine saliva collected directly from the bite or the wound bandage need to be suitable for canine STR profiling. As observed in human DNA analysis, saliva has been recovered and successfully analysed from various substrates including human skin and human bite marks [17, 18]. Canine saliva should be best recovered from the area directly surrounding the human wound, where the dog's gums and flews contacted the skin of the bitten person.
3. Severe injuries, besides being more relevant to forensic investigations, will probably also contain more canine material. The wound bandages of such cases are very likely to contain mixtures with respective quantities of human blood, which potentially has adverse effects on the ability to reconstruct the canine-specific STR profile.

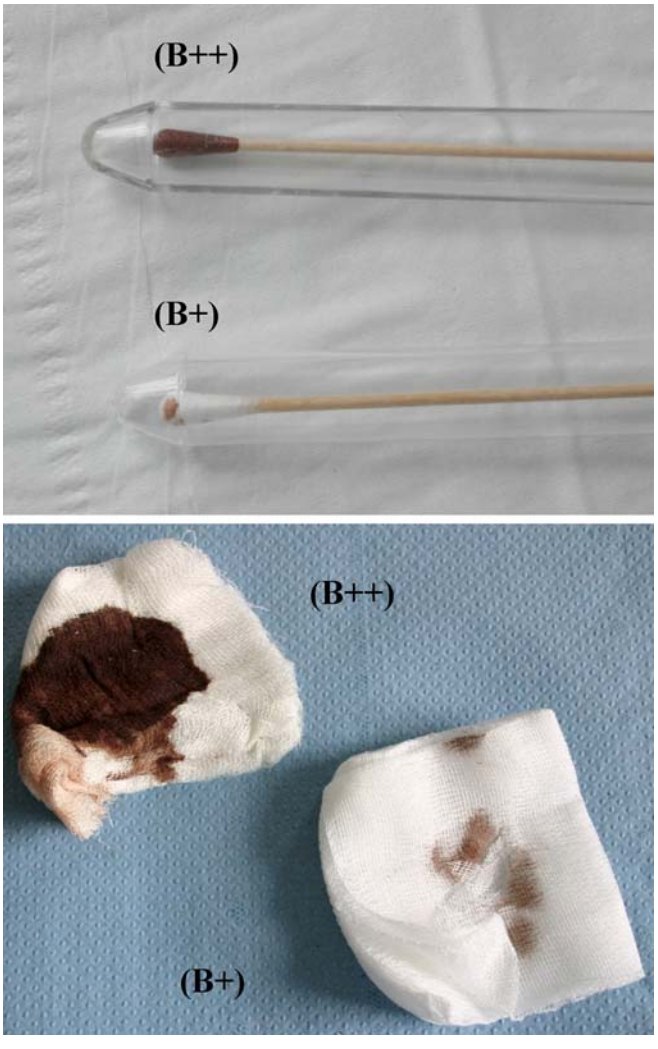

Fig. 1 Examples of bandages and swab samples as they were collected in the course of the dog bite case study. According to the amount of blood these samples were classified into (B++), (B+) and (B−, not shown)

Material and methods

DNA was extracted from buccal swabs taken from one reference dog, one reference person and from wound swabs and bandages using the phenol-chloroform method [19]. DNA concentrations of the reference samples were measured with a Hoefer DyNAQuant 200 fluorimeter using a Hoechst dye [20]. Prior to the DNA analysis of saliva recovered from the dog bite marks, the performance of canine and human STR profiling was tested using artificially prepared canine/human DNA mixtures. A DNA mixture series was prepared (Table 1) using the DNA obtained from the reference samples and was analysed with 15 canine-specific STR markers [21] and the human specific AmpFlSTR SGM Plus PCR amplification kit (Applied Biosystems AB, Foster City, CA).

Amplification, detection and analysis

The 15 canine STR markers and 2 canine-specific sex-related markers were co-amplified in 3 multiplex PCR reactions (MP1–MP3), which are described in detail in

Table 1 Mixture series of canine and human DNA analysed with 15 canine STR markers and the human specific AmpFlSTR SGM Plus PCR amplification kit (two independent analysis for each mixture)

Canine DNA (ng)	Human DNA (ng)	STR profile Dog	STR profile Human	RFUs
2	100	+	n.a.	3000
2	2	+	n.a.	3500
1	2	+	n.a.	2000
0.5	2	+	n.a.	1200
0.25	2	+	n.a.	600
0.1	2	±	n.a.	300
100	2	n.a.	+	3000
2	2	n.a.	+	4000
2	1	n.a.	+	2400
2	0.5	n.a.	+	1500
2	0.25	n.a.	+	700
2	0.1	n.a.	+	300

The average peak heights (RFUs) of the STR profiles are listed.
+ Full profile (all canine or human STRs).
± Partial profile (at least one marker dropout).
n.a. Not analysed.
RFUs Relative fluorescence units.

[21]. The total reaction volume was 50 µl including 1× PCR buffer II, 2 mM $MgCl_2$, 200 µM each dNTP, 2 U AmpliTaq Gold polymerase (AB) and 0.25 mg/ml BSA (Serva, Heidelberg). Primer concentrations were as follows:

- MP1: 100 nM (VWF.X, FH2087U, PEZ2, ZuBeCa6, SRY), and 200 nM (PEZ15, CHR.X)
- MP2: 120 nM (FH2611, ZuBeCa6), 80 nM (FH2132, ZuBeCa4), and 200 nM (FH2079, PEZ6)
- MP3: 80 nM (PEZ12, FH2611, FH2054, FH2010), and 200 nM (WILMSTF).

Thermal cycling was performed on a Gene Amp PCR System 9600 (Perkin Elmer, Norwalk, CT) comprising initial denaturation at 95°C for 11 min, 30 cycles of 94°C for 1 min, 60°C for 1 min (MP1 and MP3), 61°C for 1 min (MP2) and 72°C for 1 min and final incubation at 72°C for 60 min. The lower limit of detection of the three canine multiplexes was determined as 100 pg canine DNA for MP1 and MP3 and 250 pg canine DNA for MP2 [21]. Human-specific STRs were analysed using the AmpFlSTR SGM Plus PCR amplification kit (AB) according to the manufacturer's recommendations, with the exception of using 30 PCR cycles instead of 28. Aliquots of 2 µl of the amplification products were combined with 20 µl deionized formamide and 0.4 µl internal size standard (Genescan-500 ROX, AB), heat-denatured at 95°C for 3 min, snap-cooled on ice, and subjected to capillary electrophoresis on an ABI Prism 3100 Genetic Analyzer using POP 4, 36 cm capillary arrays and default instrument settings. The data were analysed using GeneScan Analysis version 3.7 and Genotyper version 2.5 (both AB).

Results and discussion

Canine and human DNA mixtures

As a preliminary experiment, a mixture series of canine and human DNA (see Table 1) was prepared in order to test the specificity of the canine STR primers against a high human DNA background, and to check for inhibition and/or for potential formation of artefacts during human DNA profiling in the presence of the canine-specific PCR primers.

The amplification of the canine STRs was not affected by the presence of up to 100 ng human DNA. The three canine STR multiplexes produced interpretable profiles (see Table 1) with only minor artefact peaks, none of which affected the correct interpretation of the STR profiles. An example of a canine STR profile obtained from a canine-human DNA mixture is shown in Fig. 2. We observed some pull-up peaks near the categories of the markers PEZ6 and FH2132 (middle panel), caused by bleed-through effects. In the lower panel some small artefact peaks were observed, lying outside the category range of the marker ZUBECA6.

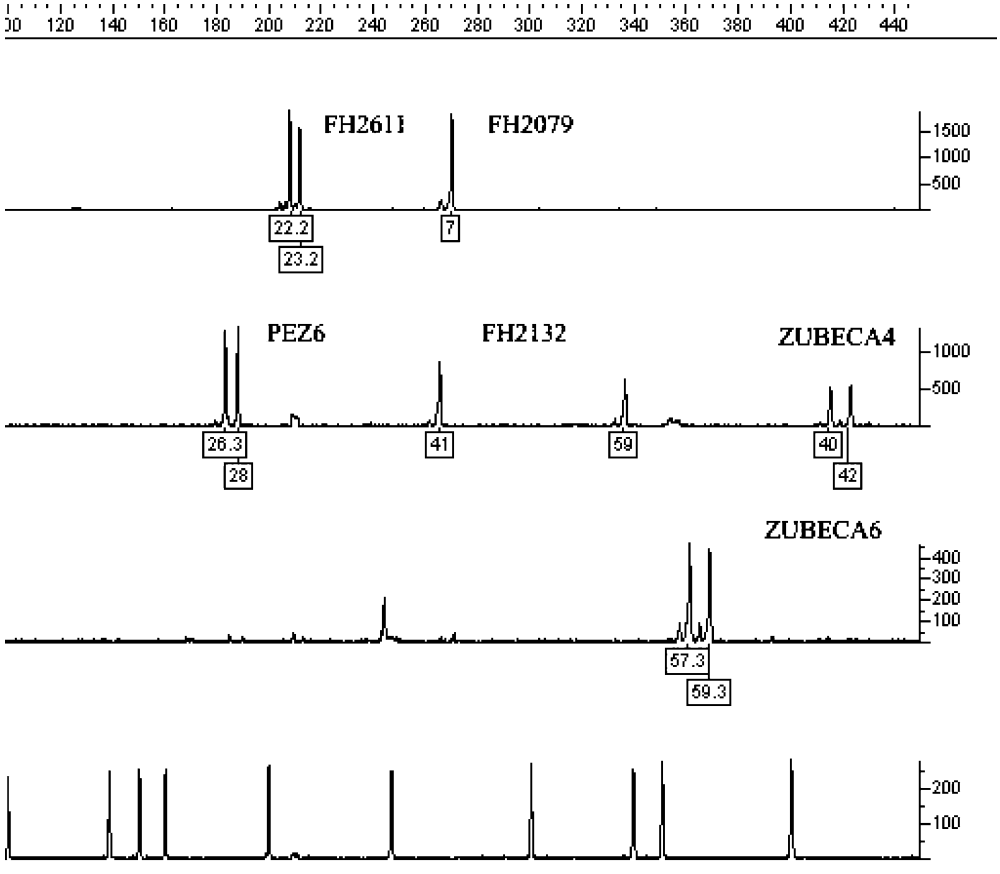

Fig. 2 Canine-specific STR profile (multiplex containing primers for 6 canine STRs) of a canine-human DNA mixture consisting of 100 ng human DNA and 2 ng canine DNA. The artefactual peaks are described in the text. Alleles were designated according to a repeat-based nomenclature as described in [22]

Amplifying human DNA in the presence of up to 2 ng of canine DNA with the AmpFlSTR SGM Plus PCR amplification kit produced the typical human-specific STR profiles (see Table 1) without occurrence of artefacts due to the canine admixture. When applying 100 ng of canine DNA, the SGM Plus kit produced a single peak (99 bp) close to the range of the gender-specific marker amelogenin. This is a commonly known artefact, which has been described for co-amplification of human and canine DNA before [16].

Dog bite case study (DBCS)

In the course of medical care, a total of 52 samples were taken from victims of dog attacks at the Department of Trauma Surgery of the University Hospital Innsbruck. Sample collection proved to be demanding because medical first aid was of paramount importance and data privacy meant that case-specific information was unavailable. However, these facts did not negatively influence the predominant methodical background of this study, namely to test canine STR profiling using samples collected directly from the dog bite itself.

The samples consisted (1) of bandages presumably used immediately after the attack to stop the blood flow which were then removed by the medical staff on admission and (2) of swabs taken from the area surrounding the wound. In 41 cases, both bandages and swabs were collected, in the remaining 11 cases either the bandages or the swabs were available (Table 2). They were grouped according to the amount of visible blood into the following 3 classes:

- (B++): large amounts of blood (Fig. 1)
- (B+): medium quantities of blood (Fig. 1) and
- (B−): no visible blood.

As human skin is a soft tissue it is unlikely that the dog hurts itself during the biting of a person, however, it cannot be completely excluded that in some cases canine blood was present on the swabs and bandages. Therefore we presume that the canine material left on the wound was in most cases canine saliva, as was confirmed by the presumptive saliva test (amylase reaction), rather than canine blood. In order to take a sample from the most promising part, the bandages were inspected visually. In a number of cases, the blood stains were partially covered with white-coloured secretions (amylase positive), which were then chosen for further DNA analysis.

The age distribution of the 52 persons examined in the course of the DBCS is shown in Fig. 3. The majority of the patients were middle-aged, 9 out of the 52 patients were children between 3 and 15 years old and 8 persons were older than 70 years: 48% of the patients were male and 52% were female.

The results of the canine STR analysis are listed in Table 2 and the success rate of obtaining specific profiles is summarised in Tables 3 and 4. Overall 30.8% of the samples gave a full canine-specific STR profile, 9.6% gave a partial profile and 59.6% showed no results.

Table 2 Collected materials and canine STR results from the 52 samples of the dog bite case study

Sample	Collected material	STRs (B)	STRs (S)	Sample	Collected material	STRs (B)	STRs (S)
1	S		±	27	B and S	-	-
2	B	±		28	B and S	-	-
3	B and S	-	-	29	B and S	+	+
4	B and S	±	±	30	B and S	-	-
5	B and S	+	+	31	B and S	+	+
6	B and S	+	+	32	B and S	-	-
7	B and S	+	+	33	S		-
8	B and S	±	±	34	B and S	-	-
9	B and S	+	+	35	B and S	-	-
10	S		±	36	B and S	-	-
11	B and S	-	-	37	B and S	-	-
12	B and S	±	+	38	B and S	-	-
13	B and S	+	+	39	B and S	-	-
14	B and S	-	-	40	B and S	-	-
15	B and S	+	+	41	B and S	-	-
16	B and S	+	+	42	B and S	-	-
17	B and S	+	±	43	B and S	-	-
18	B and S	+	+	44	B and S	-	-
19	B and S	+	+	45	B and S	-	-
20	B and S	+	+	46	S		-
21	B and S	+*	-	47	S		-
22	B and S	-	-	48	S		-
23	B and S	-	-	49	S		-
24	B and S	-	-	50	S		-
25	B and S	-	-	51	S		-
26	B and S	+	+	52	S		-

B Bandages and/or *S* swabs were collected and 15 canine STR markers were analysed.
+ Full profile (all 15 canine STRs, peak height threshold 100 RFUs).
± Partial profile (at least one marker dropout).
- No result.
*Mixture of at least two dogs.

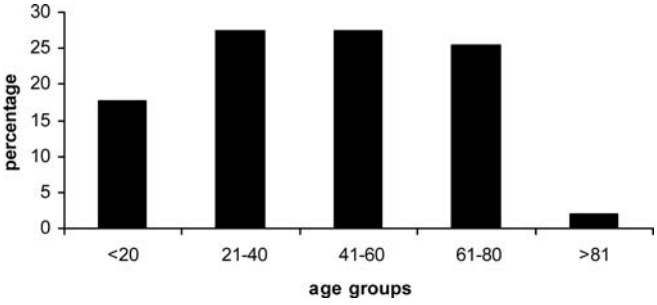

Fig. 3 Age of the victims investigated in the dog bite case study

Bandage 21 showed a mixture of at least 2 canine individuals in all 3 multiplexes (Table 2). Human DNA profiling was successful in almost all cases (94.2%), only 3 swabs (5.8%), all of which belonged to the (B−) class where no blood was detected, did not produce any results.

There seemed to be a correlation between the amount of blood on the bandage and the ability to retrieve a canine-

Table 3 Success-rate of obtaining canine-specific STR profiles (n=52)

	Full profile	Partial profile	No result
n	16	5	31
%	30.8	9.6	59.6

If a case included a bandage and a swab, the results obtained from these two different materials were summarised.

Table 4 Success rate of obtaining a useful canine-specific STR profile with respect to the type of collection device

Collected material	Class	Full profile n (%)	Partial profile n (%)	No result n (%)
Swab	B++	13 (31.7%)	2 (4.9%)	8 (19.5%)
Bandage	B++	14 (34.1%)	2 (4.9%)	8 (19.5%)
Swab	B+	1 (2.4%)	1 (2.4%)	10 (24.4%)
Bandage	B+	1 (2.4%)	1 (2.4%)	9 (22.0%)
Swab	B−	0	0	6 (14.6%)
Bandage	B−	0	0	6 (14.6%)

The results refer to the 41 cases (out of 52) where both bandages and swabs were available for analysis making a comparison of these two different collecting materials possible.
The dog bite samples were grouped into three classes according to the amount of visible blood as *(B++)* much blood,*(B+)*less blood, *(B−)* no blood.

specific STR profile. Bandages and/or swabs with a large amount of visible blood stains (B++, n=23) produced full canine profiles in 15 cases (65.2%). Samples belonging to the (B+) class (n=21) brought useful results in 5 instances (23.8%) whereas 16 (75.2%) gave no canine-specific result. No canine STR profiles were obtained from samples belonging to the (B−) class (n=8) (Fig. 4). A comparison between swab samples and bandages confirmed these findings (Table 4).

The cases described here were not selected individually but obtained from the routine casework of the Department of Trauma Surgery over a period of 1 year (between summer 2002 and summer 2003). The records of the cases are unknown. Only “first contact” swabs and bandages

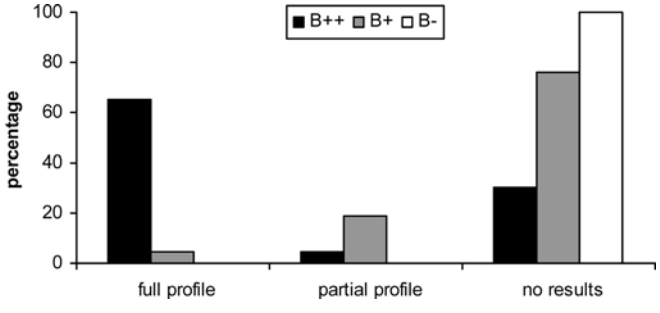

Fig. 4 Success rate of canine-specific STR profiling depending on the amount of visible blood on the sampling device. The samples collected from 52 cases were grouped into three classes: *(B++)* much blood, *(B+)* little blood, *(B−)* no blood. If a case included a bandage and a swab, the results obtained from these two different materials were summarised

from the hospital were used for DNA analysis. However, it cannot be excluded that the wounds were treated by the victims prior to the examination at the hospital. This may especially be true for the less severe attacks, as no severe wounding had been involved and the victims may have cleaned the surface of dog saliva prior to medical aid. This would explain why swabs and bandages without visible blood stains were usually less successful in terms of identifying canine-specific DNA compared with severe attacks involving relatively high amounts of human blood.

Even high concentrations of human DNA do not seem to interfere with successful canine STR typing, as indicated by the preliminary study and the samples involving large amounts of human blood. The positive correlation between the amount of human blood and canine saliva surrounding the wound—as a consequence of the intensive contact—supports the assumption that severe injuries provide a better chance of obtaining a successful canine-specific DNA result than more harmless dog bites.

Forensic approaches to investigate severe or fatal dog attacks are described in [2, 14, 15] (detailed assessment of the scene, the victim and the dog). Our results suggest that in dog biting cases where the identity of the offending dog is in question, both wound bandages and swabs serve as useful stain material in order to perform canine-specific STR analysis.

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