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CASE REPORT

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Saliva from cheese bite yields DNA profile of burglar: a case report

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Abstract Physical evidence in the form of a high quality bite mark was discovered on a piece of yellow cheese found at the scene of a crime. The cheese had been frozen by police for 10 days after recovery and before submission to the laboratory for testing. The double swab technique was used to collect DNA samples. A sample of the suspect's blood was obtained. Using PCR-based DNA typing at ten STR loci, (Profiler Plus, Perkin Elmer-Applied Biosystems) it was determined that the DNA from the cheese originated from the suspect. This case illustrates the importance of a) always considering human bite marks as both physical and biological evidence, and b) attempting DNA recovery in any case in which minute traces of saliva may be present, even in situations involving bacteria-rich foods.

Key words Forensic dentistry · Human bite mark · DNA · Saliva

Introduction

Identification of the origin of biological evidence using PCR-based DNA typing is an important modern forensic technique. Most forensic science disciplines are attempting to apply this powerful technology to any areas in which DNA evidence can be found in case work. This is true in forensic odontology in general, and in human bite mark analysis in particular. Salivary DNA evidence has been previously recovered and analyzed from inorganic substrates, such as cigarette butts [1], postage stamps [2, 3], envelopes [4] and other objects [5]. Additionally, it has been shown that saliva can potentially be recovered and typed from bite marks, sucks, fingerprints, etc. on DNA-rich substrates, such as human skin [6, 7].

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The customary approach to bite mark analysis is a physical comparison of the bite mark to known exemplars from suspects' teeth. The physical evidence from both the questioned exhibit (human skin or bitten object) and the known reference sample (study casts of teeth) must be correctly recorded to provide the best opportunity for accurate comparisons and the development of significant conclusions. When evidence from bite marks is not high quality, or physical comparison is not possible for other reasons, the importance of any salivary DNA increases.

The authors have made previous attempts to capture physical evidence from bitten foods. These attempts have not been completely successful due to problems of distortion, moisture content, flexibility and thermal sensitivity. It is difficult to produce accurate impressions and castings of bite marks in foods, such as apples, carrots and sandwiches. Finding and isolating DNA from these substances is also extremely challenging.

Case circumstances

Two suspects were apprehended within 12 h of a robbery and were found to be in possession of articles taken from a private residence. A search of the crime scene revealed no evidence which could be determined to originate from the two suspects. Circumstantial evidence existed which showed that the suspects were in possession of stolen property, but there was no evidence connecting the suspects to the actual crime scene.

With the assistance of the home owners, police recovered a small block of cheddar cheese from the scene within 36 h. It was found under a coffee table in the ransacked living room. The cheese was placed in a plastic bag and frozen at –15 °C until it was submitted for odontological examination 10 days later. When the exhibit was submitted to the laboratory, attempts were made to collect saliva which may be present on the surfaces contacted by the lips and tongue of the biter using the double swab technique [8]. One swab moistened with sterile distilled water was used to collect the saliva from the surface. A second dry swab was used to collect the moisture which remained on the surface from the first swab. The swabs were stored at –20 °C in the laboratory.

The forensic significance of the bite mark in the cheese was estimated to be very high since many details of the teeth were recorded. Static marks and dynamic striations were available for comparison since the cheese was relatively thick. It was concluded from the pattern of the teeth marks visible in the cheese that the biter exhibited a specific malocclusion (Class II, Division 2) with

severely rotated and crowded upper lateral incisors. Impressions of the bite mark were produced using President System 75 Monobody polyvinylsiloxane dental impression material (Coltene Whaledent, Mahwah, N.J.). A warrant to seize dental impressions from the suspect which exhibited a dental malocclusion and crowding of the upper teeth consistent with that in the cheese was obtained. When an attempt was made to execute the warrant, the suspect was uncooperative and refused to voluntarily provide either bite exemplars or dental impressions. It was determined that the use of force was not justified since the suspect did not have adequate opportunity to contact his legal counsel. This complication was significant since, now that the suspect knew of the interest in his teeth, it was believed that he may attempt to change the edges of the teeth by chipping or filing them, or by intentionally damaging them in an altercation with others in the remand facility.

It was decided that attempts should be made to extract DNA from the saliva swabs taken when the cheese was received at the laboratory. Theoretically, this evidence was deposited at the periphery and center of the bite mark through contact with the lips and tongue. Cheddar cheese is manufactured using mesophilic direct set cultures containing lactic acid-producing bacteria [9]. It was not known if nucleases from the bacteria-rich surface of the cheese may have resulted in DNA degradation. But, since physical comparison of the bite mark to the suspect's teeth was now thought to be problematic, the investigation was refocused in the direction of any potential biological evidence.

Material and methods

Questioned samples

The surface of the cheese contacted by the upper teeth and lips was swabbed first with a wet swab (sterile distilled water), then by a second dry swab following the double swab technique [8]. The surface contacted by the lower teeth and lips was swabbed in a similar manner. The swabs were allowed to air dry at RT for 30 min. Subsequently, the swab heads were removed from the shafts, sealed in sterile 1.5 mL tubes and stored at –20 °C pending analysis.

Each swab was placed in a separate Spin Ease tube (GIBCO BRL Life Technologies, Burlington, Canada) and 0.8 mL of lysis buffer (10 mM Tris, pH 8, 10 mM EDTA, 50 mM NaCl, 2.0% (w/v) SDS) was added with 20 µL of Proteinase K (10 mg/mL). The samples were incubated overnight at 56 °C. Each sample was submitted to organic extraction in 0.5 mL TE saturated phenol:chloroform:isoamyl alcohol (25 : 24 : 1) [10]. This was followed by a 0.5 mL n-butanol rinse [11]. The aqueous phases produced from the two swabs from the upper surface of the cheese were combined in a single tube. Similarly, the aqueous phases from the other two swabs were combined. Each of these final samples was concentrated with two passes through AMICON-100 tubes (Millipore Canada, Toronto, Canada) with filtered-autoclaved-distilled (FAD) water. The extracts were stored in a final volume of 50 µL of FAD water.

The amount of DNA was quantified by slot-blot hybridization using a D17Z1 probe [12]. PCR analysis was performed using the Profiler Plus amplification kit (PE Applied Biosystems, Foster City, CA). The amplicons were separated and detected using capillary electrophoresis (ABI-310 Genetic Analyzer, PE Applied Biosystems, Foster City, Calif.).

Known samples

Reference samples of the suspect's blood were received as bloodstains on sterile cotton gauze. A section of gauze 1 cm × 1 cm was removed and incubated overnight in lysis buffer (10 mM Tris, pH 8, 10 mM EDTA, 50 mM NaCl, 2.0% (w/v) SDS). DNA was purified from the sample using the same protocol previously described for the questioned samples. The extract was stored in a final volume of 50 µL of TE buffer (10 mM Tris, pH 8, 1 mM EDTA). A total of 1 ng of DNA from the bloodstain was amplified using the Profiler Plus kit and separation and detection was carried out using an ABI 310 Genetic Analyzer.

Genotype frequency

Statistical data and a computer application (STRquest II, Dr. G. Carmody, Ottawa, Canada) provided by the Royal Canadian Mounted Police were used to calculate the frequency of the genotype in the general Canadian population.

Results

The sample collected from the surface of the cheese which was contacted by the upper teeth and lip contained

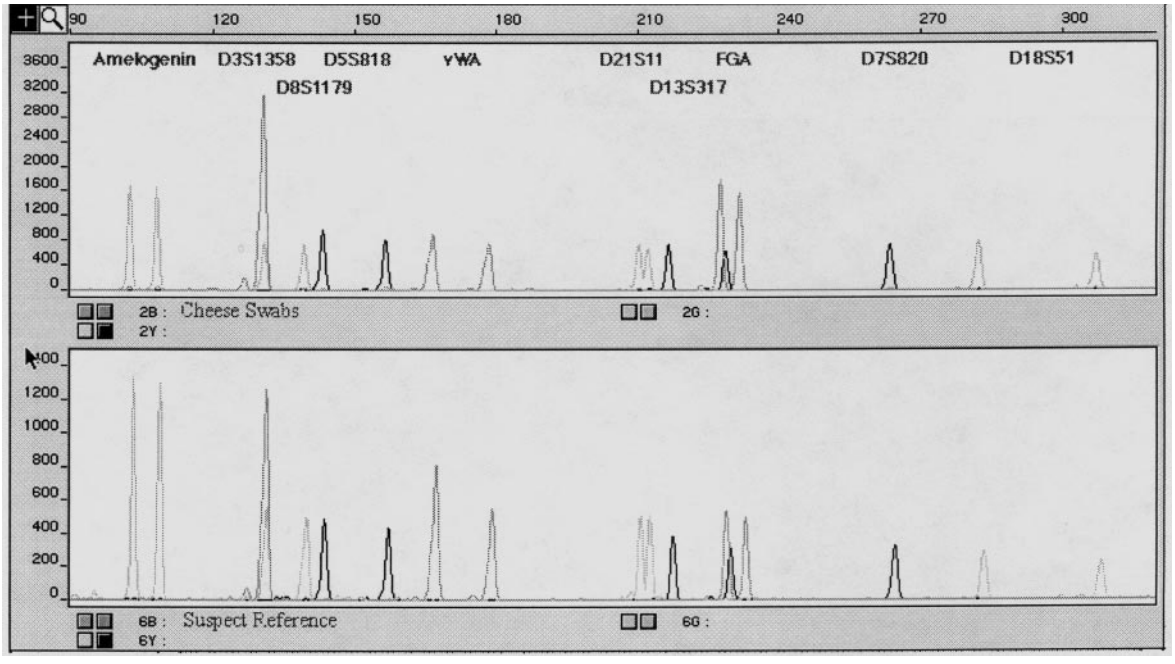

Fig. 1 Electropherograms showing comparison of DNA from cheese to DNA from suspect at 9 STR loci plus Amelogenin gender locus

Table 1 Allele calls for nine STR loci plus Amelogenin gender locus comparing DNA from cheese surface to DNA from suspect's blood

Locus	Cheese DNA	Suspect DNA
Amelogenin	X, Y	X, Y
D3S1358	17, 17	17, 17
FGA	21, 22	21, 22
vWA	14, 17	14, 17
D18S51	12, 18	12, 18
D21S11	30.2, 31	30.2, 31
D8S1179	10, 12	10, 12
D13S317	11, 14	11, 14
D5S818	10, 13	10, 13
D7S820	8, 8	8, 8

1 ng of human DNA. The sample from the surface of the cheese which was contacted by the lower teeth and lip contained 20 ng of human DNA.

Short tandem repeat (STR) profiles produced from the Profiler Plus kit were the same for the DNA recovered from the cheese and for the DNA recovered from the suspect's blood. The electropherograms for the cheese DNA and the suspect's DNA are shown in Fig. 1. The allele calls for the two profiles are shown in Table 1. Calculation of the frequency of this genotype in the Canadian population resulted in a value of 1.59×10^{-14}.

In the case described here, excellent physical evidence from a bite was found in a piece of cheese at a crime scene. However, comparison of this to the teeth of a suspect was not possible due to the technical reasons described. DNA evidence was successfully recovered from the surface of the cheese and compared to a sample of the suspect's DNA. It was determined that the origin of the DNA from the cheese and from the suspect are the same.

Discussion

It was important to consider the bitten cheese as a potential source of DNA evidence since comparison of the physical evidence to the teeth of the suspect was problematic. It was suspected that collecting saliva from the bacteria-rich surface of the cheese would be difficult, and that degradation of the biological evidence may have taken place. Theoretically, there may also be PCR inhibitors which would reduce the opportunity to identify the biter using DNA evidence.

The authors have previous experience with bitten cheese found at crime scenes. Due to improper handling of the evidence by police personnel, it has not been possible in our experience to recover and type DNA from saliva on cheese. Even the physical evidence recorded in the cheese surface may be distorted, especially by thermal changes. Attempts have been made to instruct investigators to handle biological evidence under sterile conditions and to freeze the exhibits as soon as they are recovered. In this case, the detective who found the cheese recognized its potential value and followed the suggested recovery protocol.

The double swab technique was used to collect the saliva from the cheese. In the authors' experience, this technique is superior to others for collecting saliva from human skin [8]. We thought this technique could be applied in this case to collect the traces of DNA on the cheese. DNA of sufficient quality and quantity was recovered to perform PCR-based STR typing.

Different amounts of DNA were recovered from the upper and lower surfaces of the cheese. It is unclear if this was because different amounts of DNA were present on the surfaces contacted by the upper versus the lower teeth. Amplification was successful using 200 pg of template DNA recovered from the upper surface and 1 ng of template DNA recovered from the lower surface.

It is recommended that investigators continue to think of human bite mark evidence as both physical and biological evidence. The relative importance of the DNA evidence available from saliva deposited during biting potentially increases when the physical evidence is of poor quality, or when a physical comparison is not possible. Modern PCR-based DNA typing methods provide the opportunity to test minute traces of biological evidence with a sensitivity that was previously not possible.

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