Dog registration hanging on a tooth: a case report

Registro de perros a partir de un diente: descripción de un caso

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Abstract: Dog breeder associations have regulations for the registration and breeding of their individuals. The complete dentition is between them, thus the absence of first and second molars prevents the registration of specimens. A dispute arose when the registration of a 20-month-old male dog was rejected by the Argentine club of German Shepherd Dog Breeders, because the second lower right molar was absent in routine examination. The breeder appealed the decision, alleging that the absence of the molar was accidental and not for a genetic reason, providing as evidence a molar found in his breeding kennel. Our laboratory was consulted with the objective of determining the genetic relationship between the oral swab sample obtained from the rejected dog (reference), and the molar found (evidence). For this reason, genotyping was carried out through the use of microsatellites. The results confirmed that the samples shared the same DNA profile, with the molar found being between 1.00E+11 and 1.27E+17 times more likely to have come from the dog to be registered than from an animal taken at random from the reference population. To date, there are no known reports of a similar dispute, resolved by studying a DNA profile using a tooth.

Keywords: Registration dispute, German Shepherd dog, molar tooth, microsatellites, genetic identification.

Resumen: Las asociaciones de criadores de distintas razas caninas tienen reglamentaciones para la inscripción y cría de sus individuos. La dentición completa es una de ellas, por lo que la ausencia de primeros y segundos molares impide el registro de ejemplares. Se presentó una disputa en la inscripción de un perro macho de 20 meses de edad que fue rechazada por el Club Argentino de Criadores del Perro Ovejero Alemán, debido a la ausencia del segundo molar inferior derecho. El criador apeló la decisión alegando que esta ausencia fue accidental y no por una razón genética, aportando como evidencia un molar encontrado en su criadero. Se consultó a nuestro laboratorio con el objetivo de determinar la relación genética entre la muestra de hisopado oral obtenida del animal a inscribir (referencia) y la del molar encontrado (evidencia). La genotipificación mediante el uso de

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microsatélites confirmó que ambas muestras compartían el mismo perfil de ADN, siendo entre 1,00E+11 y 1,27E+17 veces más probable que el molar encontrado procediera del perro a registrar que de un animal tomado al azar de la población de referencia. Hasta el momento no se conocen comunicaciones de una disputa similar, resuelta mediante el estudio de un perfil de ADN utilizando una pieza dentaria.

Palabras clave: Registro de perros, Ovejero alemán, molar, microsatélites, identificación genética.

Introduction

Non-human forensic genetics is the application of genetics to non-human material for the resolution and prevention of legal conflicts (Kanthaswamy, 2015). The first case reports were published about two decades ago (Giovambattista *et al.*, 2001; Savolainen & Lundeberg, 1999; Schneider *et al*., 1999). At the beginning, this discipline was applied to the investigation of crimes inherent to human beings using DNA evidence from animals, plants, bacteria and viruses (Barrientos *et al.,* 2016; Kanthaswamy, 2015). However, non-human forensic genetics has grown significantly in the last few years, extending its application to the analysis of cases of animal theft, contamination/adulteration, illegal traffic/poaching, animal attack, animal cruelty and horse doping, among others (Díaz *et al.,* 2008; Di Rocco et al., 2011; Kanthaswamy *et al.,* 2021).

Argentina is the country with the highest number of pets per capita in Latin America, with around 9 million dogs and 3 million cats, without counting stray dogs. Each year, more than 1,000 purebred dogs are exported to around 46 countries (SENASA, 2018, http://web.senasa.gob.ar), making pet breeding an important activity in Argentina. To maintain the quality of the animals bred and guarantee quality breeding to domestic and foreign buyers, the different breeder clubs have determined strict rules for the registration of animals in herd books.

The breeding and registration regulations of the Argentine club of German Shepherd Dog Breeders (Club Argentino de Criadores del Perro Ovejero Alemán, POA by its acronym in Spanish, https://clubpoa.com.ar/reglamentos/crianza-y-registro) determine that the use of dogs with absence of a first or second molar is prohibited for breeding. In addition, these animals are severely penalized in breed dog shows. Here we report a new application of non-human forensic genetics, which was used to solve a registration dispute and prevent a civil litigation.

Case report

Description

A German Shepherd breeder failed to register a 20-month-old male dog in POA because the lower right second molar of animal was absent when routine examination was carried out. The dog breeder appealed the decision, alleging that the absence of this molar was caused by an accident and not due to a genetic reason. As evidence, the breeder provided a lower right second molar tooth found in the breeding kennel. Our laboratory was consulted with the objective of determining the genetic relationship between the oral swab sample obtained from the rejected dog (reference) and the molar found (evidence).

Biological samples

Two samples were anaysed: one oral swab obtained from the German Shepherd dog (reference), and one tooth sample (evidence) provided by the breeder to demonstrate that the dog had complete dentition. Both samples were kindly provided by POA and received and analysed at the laboratory (IGEVET, UNLP-CONICET LA PLATA). POA required the owner of the animal to provide consent to report the present case, before sending the sample to IGEVET.

DNA extraction and genotyping

Before DNA extraction, the tooth was subjected to a decalcification process using a 0.5 M ethylenediaminetetraacetic acid (EDTA) - 20% N-lauroylsarcosine solution. Then, genomic DNA was purified using an in-house organic extraction method. The DNA from the oral swab sample was obtained using an extraction buffer containing 100 mM tris-hydrochloride pH 8, 10 mM EDTA, 2 M NaCl, 10% sodium dodecyl sulfate (SDS), and 10 ng/µl proteinase K. The DNA quantity and quality were measured using the Multiskan™ GO Microplate Spectrophotometer (Thermo Fisher Scientific, USA) and electrophoresis in 1% - 0.5 X tris, borate and EDTA (TBE) agarose gels.To determine if both reference and evidence samples belonged to the same dog, the obtained DNA were genotyped using the Canine Genotypes Panel 1.1 kit (Thermo Fisher Scientific) that encompasses the following 18 autosomal microsatellites: AHTk211, CXX279, REN169O18, INU055, REN54P11, INRA21, AHT137, REN169D01, AHTh260, AHTk253, INU005, INU030, FH2848, AHT121, FH2054, REN162C04, AHTh171, REN247M23, and a sex-determining locus (amelogenin). These markers are included in the 'core panel' of loci recommended by the Applied Genetics Committee of Companying Animals of the International Society for Animal Genetics (ISAG, http://www.isag.org.uk) for canine genetic identification and parentage analyses. Fragments were resolved in an Applied Biosystems 3500 sequencer and analyzed using GeneMapper®v4.1 (Thermo Fisher Scientific).

Statistical analyses

Allele frequencies of each microsatellite were calculated using the genotypes of the IGEVET database, which comprises 169 dogs from 14 breeds (including 21 German Shepherd dogs). The likelihood ratio (LR) and the random-match probability (MP) between the DNA profiles of the evidence and reference samples were calculated for each STR and for the whole set using the equations proposed by Balding & Donnelly (1995). For these estimations, 0.27 and 0.09 . values among breeds were used (Kanthaswamy *et al.*, 2009; Parker *et al.*, 2004). Furthermore, these parameters were calculated considering that the molar tooth found could belong to a relative of the declared dog, including the relatedness coefficients (k., 2k., and k.) into the formula (Weir, 2003). For these cases, 0.019 . value among kennel was used (Crespi *et al.*, 2018). All data are provided either in the text or as Supplementary material (Table S1).

Results and discussion

Comparison of the genotypes obtained from the molar and the oral swab evidenced that both samples had the same DNA profile (Figure 1). Thus, the declared dog cannot be excluded as the source of the molar tooth found in the kennel.

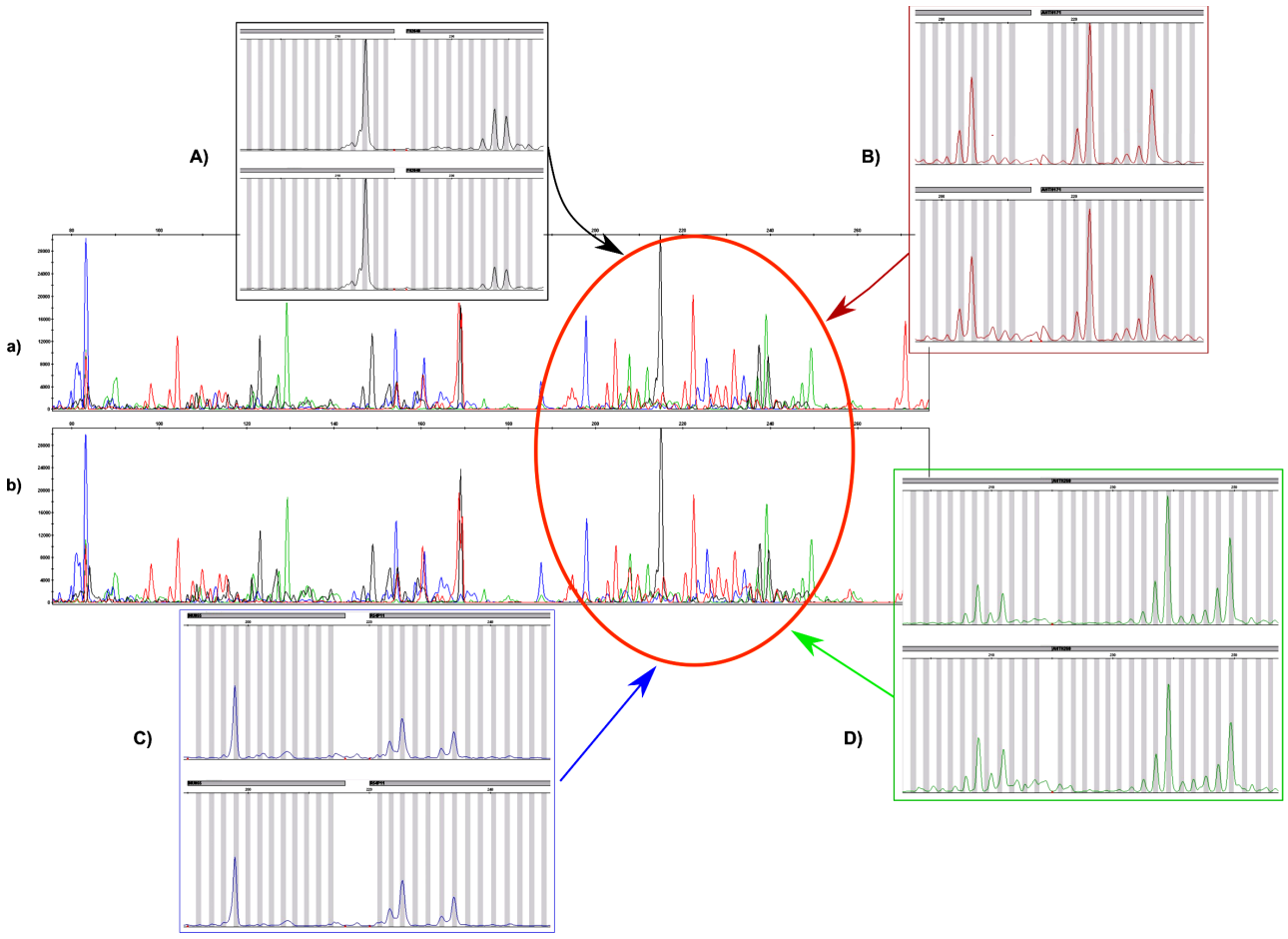

Figure 1

Electropherogram of the molar tooth found in the kennel (a) and the oral swab of the declared German Shepherd dog (b). A sector in which the fluorochromes used are identified (A: black, B: red, C: blue and D: green) is highlighted with a red circle, allowing the agreement between both samples to be observed.

In addition, the amelogenin marker confirmed the female gender of the evidence sample. The LR showed that it was between 1.00E+11 and 1.27E+17 times as likely that the tooth came from the declared German Shepherd dog than from a random unrelated animal from the dog population. Usually, relatives of dogs from different generations and litters live in a kennel (e.g., half or full siblings, parents-offspring, first cousins, uncle-nephew, and grandparent-grandchild) because dogs begin their reproductive activity very young. For this reason, a close relative of the declared dog could be the source of the tooth (Table 1).

Table 1

Random match probability and likelihood ratio estimated values for the genotypes of the molar tooth and the oral swab under different scenarios that the source of the evidence is a random unrelated animal from the population or a relative of the declared dog

Relationship	Match probability	Likelihood ratio
Unrelated[1]	9.99E-12	1.00E+11
Unrelated[2]	7.85E-18	1.27E+17
Parent - offspring[3]	2.24E-10	8.8E+07
Full siblings[3]	4.5E-09	1.4E+06
Halfsiblings[3, 4]	2.0E-12	5.1E+11
Firstuncles[3]	2.1E-13	4.7E+12

Estimated values using [1]a ϑ value of 0.27 among breeds, [2]a ϑ value of 0.09 among breeds, [3]a ϑ value of 0.019 among kennels. [4]Uncle-nephew and grandparent-grandchild had the same match probability.

As expected, these analyses showed lower values of match probability in comparison with scenarios of unrelated dog sources. However, even in the worst scenario (the source of evidence came from a full sibling) the obtained match probability would be enough with reference to the population size. These results agree with those reported by Arizmendi *et al*. (2020) in breeding kennels of Doberman Pinscher dogs, showing that the standard panels had enough discrimination power for genetic identification within a kennel.

Conclusion

To the best of our knowledge, this is the first report of a dog registration dispute between a breeder and a dog club that was solved using STR DNA profiling of a tooth,thus expanding the types of application of non-human forensic genetics.

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Conflict of interest

The authors declare that they have no conflict of interest.

Authorship statement:

Conceived the project and designed the study: JAC, EEVC, and GG. Sample collection and data acquisition: JAC, EEVC, AA, NSC, and MEZ. Analysed the data: JAC; EEVC; AA; NSC; MEZ, and MEF. Contributed to reagents/materials/analysis tools acquisition: GG, and PPG. Drafted and revised the manuscript: JAC, EEVC, NSC, MEZ, and GG. All authors have read and agreed to the published version of the manuscript.

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Supplementary material

Table S1

Gene frequencies estimated for the German Shepherd dog and the global dog populations from Argentina

Microsatélite	German Shepherd	Global dog population
AHT121		
80		0.003
92		0.051
94		0.051
96		0.181
98	0.024	0.111
100		0.087
102	0.524	0.205
104	0.048	0.117
106	0.1433	0.117
108	0.214	0.066
120	0.048	0.009
AHTH137		
131	0.417	0.474
137	0.250	0.080
141	0.250	0.158
147		0.237
149		0.026
153	0.083	0.026
AHTH171		
219		0.342
221	0.083	0.026
223	0.333	0.105
225		0.080
227		0.158
231	0.083	0.026
233	0.50	0.210
235		0.053
AHTh260		
224	0.025	0.025
234		0.003
238	0.300	0.327
240	0.225	0.041
242		0.038
244	0.050	0.176
246	0.075	0.179
248	0.050	0.050
250	0.050	0.016
252	0.225	0.101
254		0.041
260		0.003

AHTk211		
85		0.010
87	0.0	0.250
89	0.350	0.179
91	0.125	0.267
93		0.054
95	0.150	0.145
97		0.078
99	0.025	0.013
101		0.003

AHTK253		
280	0.053	0.010
282		0.003
284		0.033
286	0.132	0.242
288	0.790	0.305
290	0.026	0.202
292		0.199
294		0.007

CXX279		
114	0.083	0.184
116	0.250	0.079
118		0.026
120	0.083	0.079
124	0.333	0.316
126	0.250	0.316

FH2054		
140		0.016
142	0.050	0.012
144		0.003
148	0.200	0.053
152	0.200	0.191
154		0.016
156	0.075	0.347
160	0.050	0.047
164	0.175	0.062
168	0.225	0.181
170		0.006
172		0.041
176	0.025	0.019
228		0.003
236		0.003

FH2848		
232		0.062
234	0.125	0.094
238	0.250	0.187
240	0.250	0.062
242	0.375	0.094
244		0.500

INRA21		
89	0.125	0.033
91		0.021
93	0.125	0.134
95	0.375	0.283
97	0.125	0.051
99	0.100	0.077
101	0.050	0.265
103	0.050	0.086
105	0.050	0.039
107		0.006
113		0.006

INU005		
110	0.167	0.059
122		0.088
124	0.500	0.323
126	0.167	0.323
132	0.167	0.206

INU030		
144	0.167	0.278
146	0.083	0.028
150	0.667	0.667
156	0.083	0.028

INU055		
210	0.417	0.500
212	0.083	0.111
214	0.333	0.111
216		0.083
218	0.167	0.194

REN162C04		
200	0.200	0.100
202	0.150	0.343
204	0.025	0.187
206	0.375	0.210
208		0.100
210	0.125	0.033
212	0.125	0.027

REN169018		
154	0.028	0.003
156		0.006
158	0.278	0.070
160	0.028	0.006
162	0.111	0.256
164	0.111	0.099
166	0.306	0.074
168	0.139	0.228
170		0.189
172		0.067

REN169D01		
200		0.003
202		0.058
206		0.007
208		0.003
210	0.053	0.048
212	0.316	0.292
214	0.026	0.031
216	0.316	0.252
218	0.158	0.136
220	0.105	0.167
222	0.026	0.003

REN247M23		
268	0.187	0.389
270	0.469	0.147
272	0.187	0.389
274		0.029
276	0.062	0.020
278	0.094	0.025

REN54P11		
222		0.101
226	0.452	0.287
228		0.055
230	0.024	0.009
232	0.048	0.079
234	0.309	0.186
236	0.095	0.137
238	0.071	0.134
240		0.009
246		0.003

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