CASE REPORT

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DNA Typing in a Cattle Stealing Case

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ABSTRACT: DNA profiling was used as probative evidence in a cattle stealing case. The carcasses of the dead animals were found from a report and a farmer recognized the remains as those corresponding to the stolen animals by the farm mark on the coat. Those remains were collected as reference samples. Meat pieces were sequestered from a butchery and then sent to our Laboratory by the Justice Department of Buenos Aires (Argentine) to perform a DNA comparative analysis with the reference. Matches were found between the evidences and the references, supporting the hypothesis that the meat pieces had been obtained from the stolen animals. The butcher was suspected of stealing animals but no direct incrimination had been made yet.

KEYWORDS: forensic science, DNA typing, BoLA, microsatellites, cattle stealing

During the last decade, DNA profiling has became a widespread and well accepted methodology for pedigree analysis and paternity testing in domestic animals. Based on its accuracy, microsatellites typing has gradually replace the conventional blood and protein profiles in the assessment of cow pedigrees as well as in the certification of semen identity. Seven polymorphic microsatellites have been included in the International Comparison Test by the 1999 International Society of Animal Genetics (ISAG) where the CIGEBA group did participate.

In this report, we describe a cow stealing case where DNA profiling was used as evidence, supporting the prosecutor accusation in court. In this case, after slaughtered, the remains of the stolen animal were left by the thieves on the owner's farm. The farmer recognized the remains as those belonging to the stolen animals by the farm mark on the coat. Several pieces were then sequestered for use as reference material for further comparisons with evidences collected from the butchery (meat pieces and bones). DRB3 and DYA Bovine Leukocyte Antigen (BoLA) and six polymorphic microsatellite loci were used as genetic markers in the DNA analysis (Table 1).

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Materials and Methods

DNA Extraction

Meat pieces collected at the disposal site including limbs and skin with hair retrieve and submitted to the laboratory. The meat was in an advanced state of putrefaction. DNA was extracted according to the modified method suggested by Wagner et al. (1): 0.1 g of each sample (frozen at −80°C) were cut in small pieces with a scalpel, and suspended in 750 μL of digestion buffer (50 mM of HCl-Tris, 25 mM of EDTA, 20 mM of DTT, 2% of N-lauroylsarcosine) plus 30 μL proteinase K (10 mg/mL). The suspension was incubated overnight at 55°C. After incubation, 250 μL of 10 *M* ammonium acetate was added and the mix was centrifuged in an Eppendorf microfuge for 5 min at maximum speed. The DNA was then precipitated with Isopropanol, suspended in 200 μL of water, and then stored at −20°C until use.

Genetic Markers

DNA typing was performed by PCR amplification using the genetic markers as described in Table 1.

PCR Amplification and Genetic Analysis of PCR Products

The locus BoLA-DRB3 was typed using the primers HL030, HL031, and HL032 (2) through the heminested-PCR-RFLP method as previously described by van Eijk et al. (3). Aliquots of 12 μL of amplicons were digested with the *Hae*III, *Bsty*I, and *Rsa*I restriction enzymes in separated reactions and the restriction fragments analyzed on 6%/1X TBE (19:1) polyacrylamide minigels. BoLA-DRB3 alleles were defined according to Van Eijk et al. (3), Gelhaus et al., (4) and Maillard et al. (5).

The locus BoLA-DYA was typed using the primer HL037 and HL045 by PCR-RFLP method as previously described by Van Eijk et al. (6). Aliquots of 10 μL of amplicons were digested with the *Hind*III and *Hha*I restriction enzymes in separated reactions according to the manufacturer's recommendations. The restriction fragments were analyzed on 6%/1X TBE (19:1) polyacrylamide minigels. BoLA-DYA alleles were defined according to Skow and Nall (7).

Microsatellites—PCR was carried out in a total volume of 25 μL, containing 10 mM Tris-HCl (pH = 8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X100, 100 mM of each dNTP, 1.25 U of Taq polymerase, 0.5 mM of each primer (see Table 1), and 10 to 20

TABLE 1—*Genetic markers used in the DNA profiling test.*

Locus	Type	Number of Alleles	Chromosome	Reference
BoLA-DRB3	PCR-RFLP	32	23	(6)
BoLA-DYA	ACRS-RFLP	3	23	(6)
BM2113 (D2S26)*	MS	9	2	(12)
SPS115 (D15)*	MS	9	15	(13)
MS513 (MB026)	MS	12	23	(14)
TGLA53 (D16S3)†	MS	16	22	(14)
MGTG7 (D23S5)†	MS	10	23	(14)
INRA023 (D3S10)*	MS	12	3	(15)

* Suggested by the International Society of Animal Genetics for use for the 1999 International Comparison Test.
† Included in the FAO suggested list for biodiversity studies.

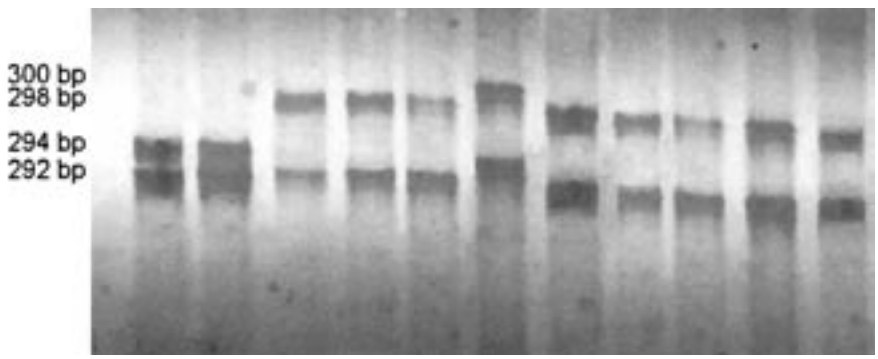

FIG. 1—*Digital image of the polyacrylamide denaturing sequencing gel developed by silver staining corresponding to* MGTG7 *microsatellites markers reported in Table 3. Line 1: Reference 1; Line 2: Evidence 1; Line 3: Reference 2; Line 4: Evidence 2; Line 5: Reference 3; Line 6: standard DNA (294 bp–300 bp); Line 7: standard DNA (292 bp–298 bp); Line 8: Evidence 3; Line 9: Evidence 4; Line 10: Evidence 5; Line 11: Evidence 6.*

ng of DNA (quantified in agarose gel by comparison with standards). The cycling conditions were: a denaturation step of 2 min at 96°C followed by 10 cycles of 1 min at 94°C, 45 sec at 58 to 63°C, and 50 sec at 72°C, and followed by 25 cycles of 1 min at 90°C, 45 sec at 58 to 63°C, and 50 sec at 72°C with a final elongation step of 5 min at 72°C. Variants were detected on 5% polyacrylamide denaturing sequencing gel by silver staining. Alleles were identified (bp size) by comparison gel mobility that corresponded to previously typed DNA's,[3] which were included in the gel as standards (see Fig. 1). In the case of MS513 microsatellite where no standard DNA were available, alleles were identified with capital letters according to the relative position of the STR fragments on the gel.

Likelihood Ratio Estimation

In such samples where the genotype matched with the references, the strength of the evidence was evaluated by calculating the conditional probability LR (8). Under the hypothesis of random match, we assume that meat pieces could have been obtained from animals, which might belong to a different breed than the reference samples. Moreover, extensive data bases representing local cow breeds are not available at this time. In order to minimize the effect of underestimation of gene frequencies, we used a 30% value for all cases, which represented the maximum mean value of the reported databases for several breeds frequencies (7,9,10).

Based on the previous estimations we used the highest reported value of inbreeding coefficient (θ) for the bovine breeds to correct deviations due to population structure.

[3] Typed DNA in the University of Illinois (generously provided by H. L. Lewin) and the 1999 International Comparison Test, where the CIGEBA group and the Blood Typing Laboratory of the "Associatión Rural del Uruguay" participated.

TABLE 2—*Likelihood ratio estimated for each locus.*

Genetic Marker	Genotype	p_1^*	p_2^*	θ	LR
MGTGT¶	292–294	0.300	0.300	0.07	5.007
	292–298	0.300	0.300	0.07	5.007
TGLA53¶	163–167	0.300	0.300	0.07	5.007
	157–167	0.300	0.300	0.07	5.007
MS513‡	A–C	0.300	0.300	0.07	5.007
	B–C	0.300	0.300	0.07	5.007
DRB3§	16–18	0.073†	0.220†	0.07	16.107
	8–18	0.169†	0.220†	0.07	9.777
DYA‖	1–2	0.300	0.300	0.07	5.007
	4–4	0.300	0.300	0.07	5.953
SPS115¶	252–252	0.300	0.300	0.07	5.953
BM2113¶	125–135	0.197	0.190	0.07	9.513
INRA023¶	206–214	0.300	0.300	0.07	5.007

* In order to avoid database effect, a mean value of 0.3 was used to estimate gene frequencies.
† Mean value of published gene frequencies.
‡ The alleles were assigned according to the relative electrophoretic mobility.
§ According to Golijow, 1996.
‖ According to van Eijk et al., 1992b.
¶ The alleles were assigned by comparing with standards.

TABLE 3—*LR estimation of the detected DNA profile.*

		Genetic Marker																
Sample Identification	Tissue Type	MGTG7		TGLA53		DRB3		DYA		BM2113		SPS115		INRA023		MS513		LR
Reference 1	Leather	292	294	163	167	16	18	1	2	ND	ND	ND	ND	ND	ND	A	C	10,123.4
Evidence 1	Bone	292	294	163	167	16	18	1	2	ND	ND	ND	ND	ND	ND	A	C	
Reference 2	Leather	292	298	157	167	8	18	4	4	123	135	252	252	206	214	B	C	1,742.396
Reference 3	Kidney	292	298	157	167	8	18	4	4	123	135	252	252	206	214	B	C	
Evidence 2	Meat piece	292	298	157	167	8	18	4	4	123	135	252	252	206	214	B	C	
Evidence 3	Meat piece	292	298	157	167	8	18	4	4	123	135	252	252	206	214	B	C	
Evidence 4	Heart	292	298	157	167	8	18	4	4	123	135	252	252	206	214	B	C	
Evidence 5	Liver	292	298	157	167	8	18	4	4	123	135	252	252	206	214	B	C	
Evidence 6	Bone	292	298	157	167	8	18	4	4	123	135	252	252	206	214	B	C	

The two DNA profiles detected were grouped.

Results and Discussion

Two different DNA profiles were detected in the reference samples (see Table 3). Both matched with the samples sequestered at the butchery: Reference 1 with Evidence 1, and References 2 and 3 with Evidences 2, 3, 4, 5, and 6, respectively (see Table 3). Both genotypes differed from those of unrelated animal included as standards. These results supported the hypothesis that the meat could have been obtained from the stolen animals.

To evaluated the strength of the evidence the likelihood ratio (LR) was calculated, weighing this hypothesis against random matching (8) and making the following assumptions:

1. In order to minimize the effect of underestimation of gene frequencies, in such cases where extensive data bases were not available, we used a 30% value for all cases, which represented the maximum mean value of the reported databases for several breeds frequencies (7,9,10) (Table 2). In the particular cases of BoLA-DRB3 and the microsatellite BM2113, we used the maximum gene frequency reported for the matching alleles (7,9).
2. Remains and meat pieces could correspond to different animals both belonging to an inbreeded population, so we adopted a $\theta = 0.07$ value, which was estimated using local highly selected breeds data base (9) analyzed with the GENEPOP PC program (11) (Table 2).

Even the butcher was suspected of dealing with stolen animals, an incriminating link had not been established until DNA profiling of the pieces of carcass where compared to DNA profiles of meat was found at the butcher's home. The results of this comparison did not exclude the possibility that the sources of meat were from the same animals, given the DNA profiles match found (LR estimated genotype probability 1 in 10,123.4 and 1 in 1,742.396, respectively).

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