CASE REPORT

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Microsatellite DNA Polymorphism Analysis in a Case of an Illegal Cattle Purchase

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ABSTRACT: A case of illegal cattle purchasing is presented. Basque country police submitted six blood samples: three from the allegedly stolen animals and three from the putative mothers. Four polymorphic DNA loci were analyzed to establish the parental relationship. From the case investigation the maternity of the alleged cattle was determined.

KEYWORDS: criminalistics, microsatellites, STR, DNA polymorphism, cattle

Traditionally, illegal animal purchasing has been difficult to prove, especially in two cases: young animals not-registered yet in the official genealogical book, and those animals not registered because they don't belong to any pure breed. Microsatellite short tandem repeat (STR) DNA polymorphism is currently used in human forensic medicine as a powerful tool to solve criminal cases with a high degree of accuracy in a short period of time. However, this practice is not common in criminal cases affecting animals of different species.

Polymorphic STR loci have been described for cattle [*1,2*], horses [*3*], swine [*4–6*], sheep [*7,8*], or dogs [*9*]. Specifically, more than 300 polymorphic loci have been described in cattle [*2*], with the aim of constructing the bovine genomic map. These loci are currently used in the detection of Quantitative Trait Loci (QTL) [*10–12*] or pedigree analysis [*13*]. In the present paper a case of illegal cattle purchase has been proven through maternal testing by using polymorphic DNA microsatellites.

Case History

Basque country police (Ertzaintza) were requested to investigate a case of alleged illegal cattle purchase from a farm located in Carranza (Bizkaia, Spain). The animals were an outcross of Charolais and the Spanish Monchina breed. Three recently dead animals were identified as the alleged stolen calves. The Ertzaintza forwarded to our laboratory six blood samples (three from the putative mothers and three from the alleged stolen calves) for analysis to establish the possible parental linkage.

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Laboratory Procedures

Sample Collection and DNA Extraction

Blood from each of the three alleged stolen animals and the three putative mothers were submitted to the laboratory (5 mL of each sample). The blood (300 μL) was centrifuged and the pellet resuspended in TE/NaCl buffer and digested with SDS/proteinase K. The DNA was obtained with a phenol:chloroform extraction procedure, followed by an ethanol precipitation and resuspended in a final volume of 300 μL in TE buffer. This genomic DNA was used for PCR Amplification.

PCR Amplification

The four loci analyzed were: BoLA DRBIII (BoLA) [*14*], ILSTS002 (IL2) [*15*], Bovine brain ribonuclease gene (BBR) [*16,17*], and Bovine TAU gene (TAU) [*18,19*], The amplifications were performed in a final volume of 20 μL in a Perkin-Elmer 48 well thermocycler. Each reaction contained about 50 ng of genomic DNA, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100 and 0.5 U AmpliTaq polymerase (Perkin-Elmer). The amplification reactions were performed as follows: first a 10 minute—95° cycle, followed by 30 cycles of 1 minute 95°C, 30 seconds 55–58°C and 40 seconds 72°C. A final cycle of 10 minutes 72° was performed. BoLA and IL2 microsatellites were amplified at 58°, BBR and TAU were amplified at 55°. Oligonucleotide primers sequence for IL2, BBR and TAU were as described [*15–19*]. The primer sequences for the BoLA locus were designed from the GeneBank (5'GCTGGTACACACTCGACCAG3', 5'GTATGGAGAGTTTCACTGGT3'). One of each pair of primers was end labelled with ^{32}P-γ- ATP. The amplification product (4 μL) was electrophoresed in a 5% polyacrylamide sequencing electrophoresis gel under denaturing conditions, and exposed to an X-ray film after drying (Fig. 1). Samples of known size (previously compared to a sequencing reaction) were used as allele size markers.

Results and Discussion

The summary of the results obtained are presented in Table 1. These data indicate that dams A, B and C can be the mothers of the stolen calves. As can be seen by the allele combination of the

TABLE 1

Animal	BBR alleles (bp)	BoLA alleles (bp)	IL2 alleles (bp)	TAU alleles (bp)
Dam A	128/140	147/151	123/125	88/94
Calf 3	140/140	147/147	115/123	88/94
Dam B	130/132	159/175	129/129	88/96
Calf 1	128/132	119/175	115/129	94/96
Dam C	128/142	125/161	125/131	94/94
Calf 2	128/130	135/161	125/131	94/94

different samples (Table 2), the only possible parentage match is: dam A/Calf 3, dam B/calf 1 and dam C/calf 2. For instance, looking at the BoLA locus, calf 3 received allele 147 bp from dam A, calf 2 received allele 161 bp from dam C and calf 1 received allele 175 bp from dam B. Any other dam/calf combination is excluded.

All the amplified loci contain simple dinucleotide repetitive elements, and their polymorphic alleles can be detected by sequencing gel electrophoresis. Allele size is given as the DNA fragment size in nucleotide base pair length (bp) (Figs. 1 and 2). In order to standardize a common protocol between all the laboratories working in this area, it would be convenient to define the allele size as the number of repetitions, as has been established by the

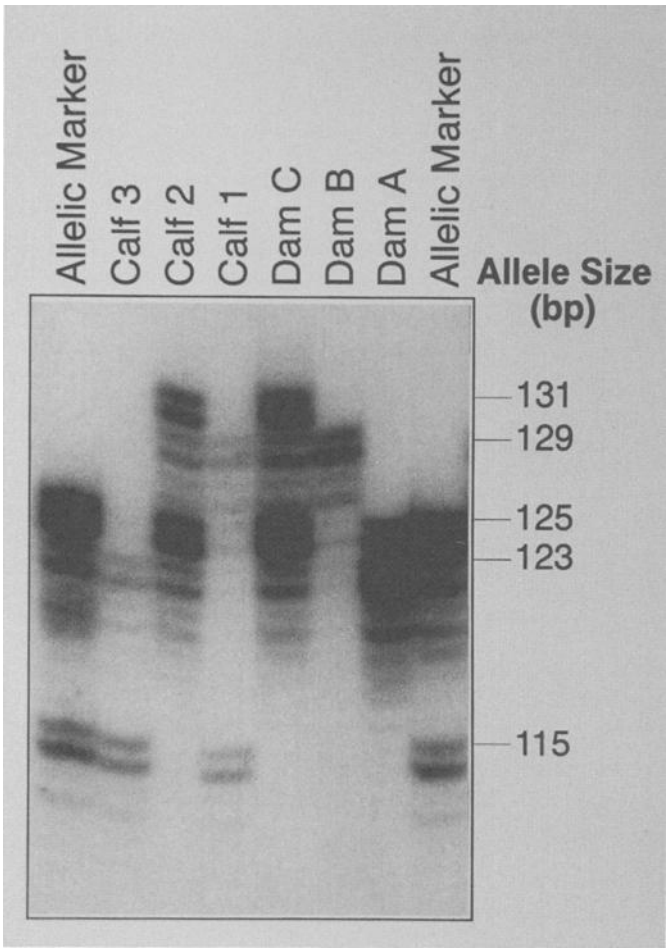

FIG. 1—*Autoradiogram of the IL2 locus amplification of the six samples. Allele sizes were defined in base pair length (bp) (see Table 1). A sample of known size, previously compared with a sequencing reaction, was used as Molecular Weight Marker.*

TABLE 2—*Shared alleles vs. exclusion.*

Dam	Calf	BBR alleles (bp)	BoLA alleles (bp)	IL2 alleles (bp)	TAU alleles (bp)
Dam A	Calf 1	128	Exclusion	Exclusion	94
	Calf 2	128	Exclusion	125	94
	Calf 3	140	147	123	88/94
Dam B	Calf 1	132	175	129	96
	Calf 2	130	Exclusion	Exclusion	Exclusion
	Calf 3	Exclusion	Exclusion	Exclusion	88
Dam C	Calf 1	128	Exclusion	Exclusion	94
	Calf 2	128	161	125/131	94
	Calf 3	Exclusion	Exclusion	Exclusion	94

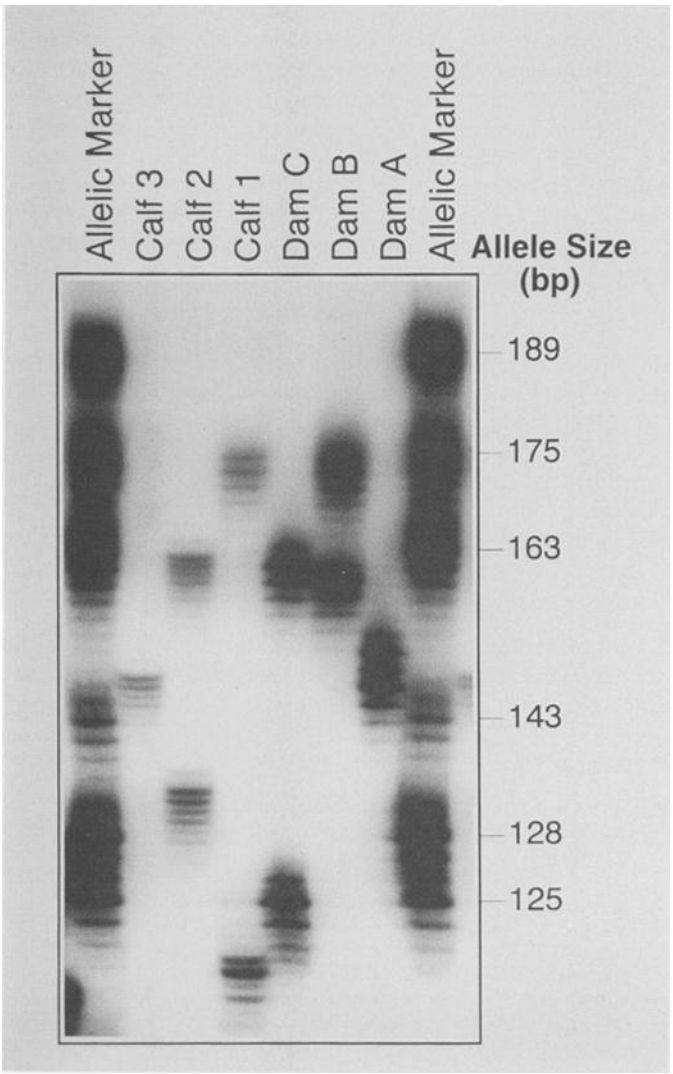

FIG. 2—*Autoradiogram of the BoLA DRB3 locus amplification of he six samples. Allele sizes were defined in base pair length (bp) (see 'able 1). Sizes were calculated as in Figure 1.*

TABLE 3—*Probability of paternity.*

Dam/Calf combination	W (Charolais)	W (Mean 5 breeds)
Dam A/Calf 3	0.9886	0.9647
Dam B/Calf 1	0.9999	0.9994
Dam C/Calf 2	0.9926	0.9984

International Society of Forensic Haemogenetics (ISFH) with the human STRs.

The calculation of the Probability of Paternity (W) and the Paternity Index (PI) was performed following the Bayesian approach [*21*]. One of the main problems found with this approach is in the definition of the specific population database to be applied. In Spain alone, more than 50 native cattle breeds have been described. The animals under investigation were not pure breed, but an outcross of Charolais and Monchina. In our laboratory, we have analyzed the allele frequency distribution of four native Spanish breeds and of the Holstein-Fresian and Charolais races [*20*]. These databases have been constructed using samples from pure unrelated individuals. In all cases the populations are in Hardy-Weinberg equilibrium. Mean Heterozygosity level in the four loci analyzed was: BBR—77%, BoLA—85%, IL2—71% and TAU 60%. Based in these data, two W were calculated (Table 3). The first one used Charolais database, and the second was the mean of the other 5 databases available. In both cases there were not significant difference in the estimate of W. W values expressed as Hummel's Verbal Predicates indicate that dam A is "very probable" the mother of calf 3, and maternity of dam B/calf 1 and dam C/calf 3 are "practically proven."

This type of analysis can be applied to other animal species as swine, horses, sheep or dogs. For instance, another case of illegal purchasing, this time in dogs, was submitted by the Ertzaintza to our laboratory. We used four loci and excluded the alleged mother.

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