MICROSATELLITE DNA POLYMORPHISM ANALYSIS IN A CASE OF ILLEGAL CATTLE PURCHASE

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INTRODUCTION

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 stablish the possible parental linkage.

LABORATORY PROCEDURES

SAMPLE COLLECTION AND DNA EXTRACTION: 5 ml of blood from each of the three alleged stolen animals and the three putative mothers were submitted to the laboratory. 300 µl of blood were 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.

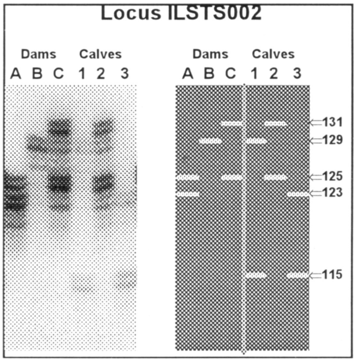
PCR AMPLIFICATION: The four loci analyzed were: BoLA DRBIII (BoLA) [14], ILSTS002 (IL2) [15], Bovine brain ribonuclease gene (BBR) [16,17], and Bovine TAU gene (TAU) [18,19]. BoLA/IL2 and BBR/TAU loci were coamplified. Oligonucleotide primers sequence for IL2, BBR and TAU were as described [15-19]. The primer sequences for the BoLA locus were designed from the GeneBank. One of each pair of primers was end labelled with ^{32}P-γ- ATP. 4 μl of the amplification product were electrophoresed in a 5% polyacrilamide 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 I. These data indicate that dams A, B and C can be the mothers of the stolen calves. As can be inferred by the allele combination of the different samples, 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.

TABLE I

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**



The calculation of the Probability of Paternity (W) and the Paternity Index (PI) was performed following the Bayesian approach. 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 4 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. Based in these data, two W were calculated (Table II): 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 damB/calf1 and damC/cald3 are "practically proven".

TABLE II
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

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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