



Paternity in Brazilian goats through the use of DNA microsatellites

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ABSTRACT - A total of 292 animals from three breeds (Alpine and Saanen dairy breeds, and the Brazilian naturalized breed Moxotó) were genotyped, comprising 276 paternity cases. Statistical analyses were carried out by using TFPGA and CERVUS programs. Heterozygosity ranged from 0.542 (*ILSTS005*) to 0.825 (*INRA006*), with an average of 0.717 for all loci. Polymorphic information content (PIC) was 0.676 and 0.542, and combined exclusion probabilities (EP) were 0.999591 and 0.988375 for known and unknown maternal genotypes, respectively. The microsatellite system reveals 10% of paternity misidentification in evaluated registers.

Key Words: Brazil, local breed, paternity exclusion, pedigree mismatching, polymorphism

Paternidade em caprinos por meio de microssatélites de DNA

RESUMO - Um total de 292 animais de três raças (leiteiras Alpina e Saanen e a raça brasileira naturalizada Moxotó) foram genotipados, compreendendo 276 casos de paternidade. As análises estatísticas foram realizadas nos programas TFPGA e CERVUS. A heterozigosidade esperada variou de 0,542 (*ILSTS005*) a 0,825 (*INRA006*), com média de 0,717 para todos os *loci*. O conteúdo de informação polimórfica (*Polymorphic information content*, PIC) para todos os *loci*, foi de 0,676 e 0,542 e a probabilidade de exclusão (EP), de 0,999591 e 0,988375 para casos em que o genótipo materno era conhecido e desconhecido, respectivamente. A taxa de erro de identificação de paternidade é de 10% nos registros avaliados.

Palavras-chave: Brasil, erro de *pedigree*, exclusão de paternidade, polimorfismo, raça naturalizada

Introduction

Goats are the most widely spread domestic species in the world and play an important economical role in developing countries. Brazil has the tenth largest world goat herd with about ten million animals (IBGE, 2006). In Brazil, goat breeding ranges from an intensive system in the dairy basins in the southern and southeastern regions to an extensive raising system at the Northeast region, where a large number of herds is found, contributing to sustainable small scale agriculture. Goat genetic resources are very diverse, with foreign European breeds like Alpine and Saanen being the most important resources for dairy production. On the other hand, at the Northeast region, a large number of herds has no defined breed, or is composed by naturalized groups, such as Moxotó, Canindé, Marota, and Repartida (Machado, 2000).

There isn't an official National Genetic Goat Improvement Program in Brazil. Genealogical data are

registered at the Associação Brasileira de Criadores de Caprinos (ABCC) and its branches. The few initiatives to create a goat database are isolated efforts of breeders and research institutions without substantial results. All breeding programs are based on non official information and considering the losses on selection accuracy and on genetic gain generated by genealogical misidentification the need for a correct paternal identification is a major concern for the evaluation of genetic parameters and the success of breeding programs.

Statistical methods for pedigree checking in natural populations or those on which data are taken on a large scale may differ in focus from commercial laboratories, whose goal is to confirm genetic compatibility of the alleged father, mother and progeny trio using parentage exclusion. The goal of the present study is to use eleven microsatellites loci to solve genealogy and to verify the accuracy of the registered pedigrees in Brazilian goat herds.

Material and Methods

Pedigree register for 121 Alpine and 94 Saanen goats from Universidade Federal de Viçosa, 36 Alpine and 19 Saanen goats from a private herd, and 22 Moxotó goats from preservation herd of Embrapa, were analyzed. Samples were distributed in existing sire families of each herd to obtain a progeny group for each male breeding stock. Known mothers were genotyped within the herd.

For Alpine and Saanen breeds, blood was collected from the jugular vein, using a vacuum system, and stored at 4°C for 6 h. DNA was extracted by following the phenol:chloroform protocol (Sambrook et al., 1989). DNA samples from the Moxotó herd were obtained from hair follicles, following the cetiltrimetilammonium bromide protocol, as described by Ferreira & Grataplagia (1998).

The following loci were tested: *BETACAP*, *INRA005*, *ILSTS087*, *INRA006*, *INRA063*, *INRABERN172*, *ILSTS005*, *ILSTS011*, *SRCRSP05*, *OARFCB48*, and *BM3205* (Table 1). The *BETACAP* locus belongs to the casein gene cluster (Pépin et al., 1995). *INRA005*, *ILSTS0087*, *INRA006*, *INRA063*, *INRABERN172*, *ILSTS005*, *ILSTS011*, and *BM3205* loci in goats are homologous to cattle, and the *OARFCB48* locus is homologous to sheep (Vaiman et al., 1996). Arevalo et al. (1994) described the *SRCRSP005* locus in goats. The primer sequence of these loci can be accessed in GOATMAP DATABASE (<http://locus.jouy.inra.fr>).

Each 20 µl PCR reaction contained 25 ng DNA, 20 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.2 mM each dNTP, 1.25 mM to 2.5 mM MgCl₂, and 1 U Taq DNA polymerase. For each one of the different loci, primer concentrations were adjusted between 0.12 µM and 0.20 µM. The denaturation step of 94°C/3 min was followed by 27 cycles at 94°C/1 min, annealing

temperature according to each primer pair/1 min, 72°C/1 min, and a final extension of 72°C/20 min.

Reverse primers were fluorescently labeled with Tet, 6-Fam, and Hex dyes, so amplified fragments did not overlap, and multiplexed electrophoresis could be used. Amplified fragments were submitted to capillary electrophoresis at 9 V, 60°C for 22 minutes and analyzed with ABI 310 *Genetic Analyzer* from *Applied Biosystem*TM, utilizing *Genescan*[®] software (Table 1).

Gene frequencies were obtained for each allele by direct count. The polymorphic information content was used to quantify polymorphism when one progenitor genotype was known (PIC₁) (Bolstein et al., 1980) and when both progenitors genotypes were unknown (PIC₂) (Da & Lewin, 1995).

Exclusion probability was calculated with TFPGA software (Miller, 1997), firstly for cases in which one progenitor was confirmed, and genotypes were known for the alleged father, mother and progeny (EP₁), and secondly when just the father and progeny genotypes were known (EP₂) (Jamieson & Taylor, 1997).

The maximum likelihood method by the CERVUS program (Marshall et al., 1998) was applied to check pedigree mismatching. CERVUS program is used to calculate allelic frequencies in a simulation approach and to determine the confidence in parentage assignments by calculating critical values of the log likelihood statistics Δ (Delta).

The exact test of Hardy-Weinberg proportion was performed by TFPGA software (Miller, 1997), using the method developed by Guo and Thompson (1992) of Markov Chain (10 batches, 5.000 permutations, and 1.000 dememorization steps).

Table 1 - *Locus*, chromosome localization, annealing temperature, MgCl₂ concentration, primer concentration, dye, number of alleles observed, and allele size

Locus	Chromosome	Annealing temperature (°C)	MgCl ₂ concentration (mM)	Primer concentration (µM)	Dye	Number of alleles observed	Allele Size (pb)
BETACAP	6	55	1.25	0.16	Tet	7	160-174
INRA005	12	55	1.25	0.16	6-Fam	5	113-121
ILSTS0087	n	55	1.25	0.12	6-Fam	9	135-153
INRA006	3	55	2.50	0.16	Hex	10	106-126
INRA063	18	56	2.0	0.20	6-Fam	7	162-207
INRABERN172	26	55	1.25	0.20	Hex	8	136-152
ILSTS005	10	56	2.0	0.16	Tet	7	175-187
ILSTS011	14	56	2.0	0.16	6-Fam	10	262-280
SRCRSP05	21	56	2.0	0.16	Hex	7	157-173
OARFCB48	17	58	3.0	0.20	Tet	7	152-164
BM3205	1	50	1.25	0.20	Hex	11	215-237

n *locus* no genetic or RH mapped.

Results and Discussion

Expected average heterozygosity (HE) was 0.717, ranging from 0.542 (*ILSTS005*) to 0.825 (*INRA006*). As expected, polymorphic information content was greater for loci with greater heterozygosity (*INRA006*). Mean allele number per locus was 7.18 for Alpine and Saanen breeds and 3.5 for the Moxotó breed.

The polymorphic information content (when one progenitor genotype was known and when both progenitor genotypes were unknown), and the probability of combined exclusion were 0.676 and 0.5420; and 0.999591 and 0.988375, respectively, when the maternal genotype is known or not (Table 2). In goats, Luikart et al. (1999) developed a panel with 22 microsatellites in two multiplex reactions to test paternity and obtained an exclusion probability >0.99999. In Brazil, Curi & Lopes (2002) achieved a combined exclusion probability of 0.9789 with nine bovine microsatellite loci. These obtained exclusion probabilities must be improved by more loci to attend the paternity test. However, the microsatellites tested are informative by scan registers errors and quantified some noise in genetic evaluation in Brazil.

The chosen loci are independent, located on distinct chromosomes and have simple Mendelian inheritance. Exclusion of the panel was similar to that proposed by Luikart et al. (1999) for the Saanen breed in Switzerland. *BETACAP*, *INRA005*, *ILSTS005*, and *SRCRSP05* loci are moderately polymorphic ($H_E > 0.70$) as considered by Ott (1992). Removal of each one of the above loci decreased exclusion probability for unknown mother

genotype respectively to 0.9846, 0.9854, 0.9862, and 0.9835. Removal of all four loci decreased exclusion probability for unknown mother genotype to 0.9676.

Twenty-seven father-progeny genotypes had at least three mismatching genotypes which were considered as register errors in the paternity exclusion test corresponding to approximately 10% of the total. Additionally, 39 cases remained unsolved by the exclusion approach (with 1-3 supposed father-progeny incompatibilities). In these remaining cases, either the alleged father could have been rejected as the real father or other statistical proxies can be used (Marshal et al., 1998).

Paternity register error in the present study was close to 10%, which is similar to the rates in European cattle dairy breeds (Ron et al., 1996). In Brazil, Rosa et al. (1997) obtained approximately 15% error in Nelore cattle, and Curi and Lopes (2002) observed 27.5% error in Gir families registered in the herd book of Associação Brasileira dos Criadores de Zebu (ABCZ).

Significant deviations ($p < 0.01$) from Hardy-Weinberg balance were verified for *INRA063* and *OARFCB48* loci in all breeds, and it is the same loci responsible for the greatest number of genotyping mismatches with frequencies of 0.1571 and 0.080, respectively.

The deviation of the Hardy-Weinberg verified in this study for some loci can be caused due to sampling (Wahlund effect), having to reach the balance when more flocks are used (Luikart et al., 1999). Null alleles were verified for *INRA063* and *OARFCB48* loci, leading to a deficit of observed heterozygote. Null or non-amplified alleles could also be the reason for the Hardy-Weinberg imbalance.

Table 2 - Number of observation, expected heterozygosity, polymorphic information content and exclusion probability for all loci under study

Loci	Number of observation	Expected heterozygosity	Polymorphic information content ¹	Polymorphic information content ²	Exclusion probability ¹	Exclusion probability ²
BETACAP	288	0.663	0.606	0.462	0.409	0.247
INRA005	289	0.617	0.560	0.414	0.362	0.203
ILSTS0087	290	0.742	0.702	0.568	0.524	0.347
INRA006	290	0.825	0.802	0.689	0.656	0.484
INRA063	287	0.713	0.665	0.525	0.475	0.303
INRABERNI72	289	0.772	0.739	0.610	0.568	0.387
ILSTS005	286	0.542	0.480	0.338	0.294	0.154
ILSTS011	285	0.765	0.732	0.600	0.558	0.377
SRCRSP05	283	0.690	0.656	0.514	0.477	0.294
OARFCB48	248	0.792	0.757	0.631	0.584	0.406
BM3205	240	0.771	0.738	0.610	0.570	0.392
All loci	292	0.717	0.676	0.542	0.99959	0.98838

¹ Genotype of the mother was known.

² Genotype of mother was unknown.

Conclusions

The eleven microsatellite loci compose a panel able to check accurately pedigrees. Knowledge of the maternal genotype greatly increases test accuracy, thus whenever it is possible, genotype of the dam has to be accessed. Modifications made over time may be implemented to incorporate other loci to improve exclusion probability, especially when the maternal genotype is unknown.

References

- AREVALO, E.; HOLDER, D.S.; DERR, J.N. et al. Goat microsatellite dinucleotide repeat polymorphisms at the SR-CRSP 1, 2, 3, 4 and 5 loci. **Animal Genetics**, v.25, p.202, 1994.
- BOLSTEIN, D.; WHITE, R.L.; SKOLNICK, M. et al. Construction of genetic linkage map in man using restriction fragment length polymorphism. **American Journal of Human Genetics**, v.32, p.314-331, 1980.
- CURI, R.A.; LOPES, C.R. Evaluation of nine microsatellite loci and misidentification paternity frequency in a population of Gyr breed bovines. **Brazilian Journal of Veterinary Research Animal Science**, v.39, p.129-135, 2002.
- DA, Y.; LEWIN H. A. Linkage information content and efficiency of full-sib and half-sib designs for gene mapping. **Theoretical and Applied Genetics**, v.90, p.699-706, 1995.
- GUO, S.W.; THOMPSON, E.A. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. **Biometrics**, v.48, p.361-372, 1992.
- INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA - IBGE. **Censo Agropecuário de 2001**. Disponível em: <<http://www.ibge.org.br>> Acesso em: 20/1/2004.
- JAMIESON, A.; TAYLOR, ST. C.S. Comparisons of three probability formulae for parentage exclusion. **Animal Genetics**, v.28, p.397-400, 1997.
- FERREIRA, M.A.; GRATTAPAGLIA, D. **Introdução ao uso de marcadores moleculares em análise genética**. 3.ed. Brasília: Embrapa Cenargen, 1998. p.220.
- LUIKART, G.; BIJU-DUVAL, M-P.; ERTUGRUL, O. et al. Power of 22 microsatellite markers in fluorescent multiplexes for parentage testing in goats (*Capra hircus*). **Animal Genetics**, v.30, p.431-438, 1999.
- MACHADO, T.M.M. Caprins autochtones du Brésil: L'identification, la standardisation et la sauvegarde. In: INTERNATIONAL CONFERENCE ON GOATS, 7., 2000, Tours. **Proceedings...** INRA, 2000. v.2, p.941-943.
- MARSHALL, T.C.; SLATE, J.; KRUK, L.E.B. et al. Statistical confidence for likelihood-based paternity inference in natural populations. **Molecular Ecology**, v.7, p.639-655, 1998.
- MILLER, M.P. [1997]. **TFPGA- Tools for populations genetics analyses**. V 1.3 A Window program for the analysis of allozyme and molecular population genetic data. Disponível em: <<http://herb.bio.nau.edu/~miller/tfpga.htm>> Acesso em: 20/11/2005.
- OTT, J. Strategies for characterizing highly polymorphic markers in human gene mapping. **American Journal of Human Genetics**, v.51, p.283-290, 1992.
- PEPIN, L.; AMIGUES, Y.; LEPINGGLE, A. et al. Sequence conservation of microsatellites between cattle, goat and related species. **Heredity**, v.74, p.53-61, 1995.
- ROSA, A.J.M.; PACKER, I. U.; REGITANO, L.C.A. Caracterização da raça Nelore e teste de paternidade por marcadores moleculares. In: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 34., 1997, Juiz de Fora. **Anais...** Juiz de Fora: Embrapa, 1997. v.3, p.62-64.
- RON, M.; BLANC, Y.; BAND, M. et al. Misidentification rate in Israeli dairy cattle population and its implications for genetic improvement. **Journal of Dairy Science**, v.79, p.676-681, 1996.
- SAMBROOK, J.; FRITSCH, E.F.; MANIATS, T. **Molecular cloning: a laboratory manual**. 2.ed. New York: Cold Spring Harbor Lab, 1989. 479p.
- VAIMAN, D.; SCHIBLER, L.; BOURGEOIS, F. et al. A genetic linkage map of the male goat genome. **Genetics**, v.144, p.279-305, 1996.