# BIOCHEMICAL POLYMORPHISMS IN SHEEP AND THEIR POTENTIAL USE FOR PATERNITY TESTS.

# POLIMORFISMOS BIOQUIMICOS EM OVINOS E A POSSIBILIDADE DE SUA UTILIZAÇÃO EM TESTES DE PATERNIDADE.

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## SUMMARY

The genetic variability of 22 protein loci was investigated in two sheep flocks: 22 females Romney Marsh and 124 animals derived from crossbreeding between Romney Marsh and Merino Booroola, reared by the Brazilian Agricultural Research Corporation (Empresa Brasileira de Pesquisa Agropecuária - EMBRAPA, Bagé, RS, Brazil). Eight loci were polymorphic; the others showed no variation. The usefulness of the eight plymorphic systems (Cat, DIA I, EP-1, EsA, HbB, ME, Tf, and X Prot.) in parentage tests was analyzed. The probability to find two random identical animals in each breed was estimated as 1:1000. The efficiency of these proteins for exclusion of one of two possible sires in parentage tests was about 77% both for Romney Marsh and Romney/ Booroola flocks. Although parentage tests in sheep have not been enforced in Brazil up to now, the establishment of this technique is important for the prevention of non-paternity on the excellent rams.

**Key words:** Sheep, genetic polymorphisms, paternity tests.

## RESUMO

Investigou-se a variabilidade genética em 22 locos proteicos em uma amostra de 22 fêmeas Romney-Marsh e em 142 animais resultantes do cruzamento entre Romney-Marsh e Merino Booroola, criados pela Empresa Brasileira de Pesquisa Agropecuária (EMPRAPA, Bagé, RS, Brasil). Oito destes sistemas mostraram-se polimórficos, os demais não apresentando variação. Avaliou-se a eficiência destes oito sistemas proteicos polimórficos (Cat,DIA I, EP-1, EsA, HbB, ME, Tf e Prot. X ), no controle de filiação. A probabilidade de identidade entre dois animais retirados ao acaso da população foi estimada em 1:1000 em cada rebanho. A eficiência destes marcadores genéticos em excluir um entre dois possíveis pais foi de cerca de 77% tanto para os

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animais Romney Marsh como para os Romney/ Booroola. Testes de controle de paternidade, em ovinos, não têm sido realizados, até agora, no Brasil, mas o estabelecimento desta técnica seria importante para assegurar a paternidade da descendência de reprodutores zootecnicamente superiores.

**Palavras-chave:** Ovinos, polimorfismos genéticos, testes de paternidade.

## INTRODUCTION

A correct identification of farm animals is important to assure the paternity of animals presumed to be offspring of selected sires, for artificial insemination and to identify donors in embryo transfer programs. Blood genetic polymorphisms are useful tools for this purpose, mainly due to their monogenic inheritance and the fact that they can be precise and easily identified.

Many polymorphisms have been described in sheep (TUCKER, 1971; MANWELL & BAKER, 1977); however, their utilization in pedigree identification is restricted to some foreign countries (HALL, 1975; HOJNY & STRATIL, 1978), with no references in Brazil. In the present investigation we determined the efficiency of some protein systems for parentage control in two sheep flocks from Rio Grande de Sul, Brazil.

## MATERIAL AND METHODS

The genetic variability at 22 loci was investigated in a sample of 22 females from a Romney Marsh flock and 124 animals derived from crossbreeding between Romney Marsh and Merino Booroola, reared by the Brazilian Agricultural Research Corporation (Empresa Brasileira de Pesquisa Agropecuária -EMBRAPA, Bagé, RS, Brazil).

Blood samples were obtained by puncture of the jugular vein, placed in tubes with ACD, refrigerated shortly after collection and immediately carried to the laboratory, where the red cells were separated, washed in saline and glycerolized according to WEIMER et al. (1981). Plasma and red cells were stored at -20°C until the tests, which were mainly performed by electrophoresis.

The following proteins were typed using methods described or referenced elsewhere (HENKES et al.,1994): acid phosphatase - ACP, albumin - Alb, arylesterase - EsA, carbonic anhydrase - CA, catalase-Cat, ceruloplasmin - Cp, erythrocyte protein 1 - EP-1, esterases B and D - EsB and EsD loci, glucose phosphate isomerase - GPI, glucose-6-phosphate dehydrogenase - G6PD, glyoxalase I - GLO, hemoglobin - HbA and HbB loci, malic enzyme - ME, NADH diaphorase - DIA I and DIA II loci, phosphogluconate dehydrogenase - PGD, slow alpha-2 macroglobulin, superoxide dismutase - SOD, transferrin - Tf and X protein - X Prot.

Allelic designations were assumed from published descriptions except for Alb and Tf which were compared with international standards.

Gene frequencies were calculated by the gene-counting method for the codominant systems or by assuming Hardy-Weinberg equilibrium for the dominant loci.

The probability that two individuals chosen at random in a population will have the same genotype was calculated according to VAN ZEVEREN et al. (1990) and the exclusion probabilities of an incorrectly alleged parent were calculated according to OISHI & ABE (1970, 1975).

For the crossbred sheep sample we had complete genealogic information; in these cases we checked the assigned parentage to compare the expected and observed exclusion probabilities of each protein system. When exclusion was observed the protein were retested with the samples from sire, dam and lamb side by side on the same electrophoresis gel to discard laboratory errors.

## RESULTS AND DISCUSSION

Table I shows the gene frequencies observed in eight polymorphic systems in both flocks. The other proteins were all monomorphic. In general, the degree of variability was similar to those described thus far for other sheep breeds (TUCKER,1971; MANWELL & BAKER,1977).

Considering the eight polymorphic systems, the estimate of the probability that two individuals chosen at random will have similar genotypes was made and gave a value 0.001 for both flocks. This result suggests a good efficiency of these systems in identifying animals since the odds of finding at random two identical animals is highly improbable for both flocks.

Table II reports the single and combined probabilities of exclusion of one of two possible sires in parentage tests in the studied flocks.

According to GÜNDEL & REETZ (1981), the probability of detecting false parents depends on the number of systems investigated, on the number of alleles per system, and on the allele frequencies in the

Table I - Allele frequencies for eight protein loci in two sheep flocks.

| Loci | Alleles | Gene Frequencies | |
|---|---|---|---|
| | | Romney | Merino Booroola |
| HbB | **HbB A** | 0.26 | 0.51 |
| | **HbB B** | 0.74 | 0.49 |
| ME | **ME F** | 0.93 | 0.86 |
| | **ME S** | 0.07 | 0.14 |
| Cat | **Cat F** | 0.25 | 0.47 |
| | **Cat S** | 0.75 | 0.53 |
| DIA I | **DIA I F** | 0.43 | 0.40 |
| | **DIA I S** | 0.57 | 0.60 |
| EP-1 | **EP-1 A** | 0.55 | 0.42 |
| | **EP-1 B** | 0.45 | 0.58 |
| X | **X +** | 0.26 | 0.12 |
| | **X -** | 0.74 | 0.88 |
| EsA | **EsA +** | 0.23 | 0.06 |
| | **EsA -** | 0.77 | 0.94 |
| Tf | **Tf A** | 0.43 | 0.57 |
| | **Tf B** | 0.07 | 0.14 |
| | **Tf C** | 0.32 | 0.24 |
| | **Tf D** | 0.18 | 0.04 |
| | **Tf E** | 0.00 | 0.01 |

Table II - Estimation of the average probability (P) of exclusion of one of two possible sires in parentage analysis using eight protein loci.

| Systems | Romney / Booroola | | Romney Marsh | |
|---|---|---|---|---|
| | No. alleles | P | No. alleles | P |
| HbB | 2 | 0.1874 | 2 | 0.1554 |
| ME | 2 | 0.1059 | 2 | 0.0609 |
| Cat | 2 | 0.1870 | 2 | 0.1523 |
| DIA I | 2 | 0.1824 | 2 | 0.1850 |
| EP-1 | 2 | 0.1843 | 2 | 0.1862 |
| X Prot | 2 | 0.0720 | 2 | 0.0780 |
| EsA | 2 | 0.0468 | 2 | 0.0808 |
| Tf | 5 | 0.3481 | 4 | 0.4106 |
| All loci | - | 0.7728 | - | 0.7772 |

Table III - Five cases of parentage exclusion observed.

| Case | Systems | Phenotypes | | |
|---|---|---|---|---|
| | | Sire | Dam | Lamb |
| 1 | HbB | AA | AB | BB |
| 2 | EsA | - | - | + |
| 3 | EP-1 | BB | AA | BB |
| 4 | X Prot | - | - | + |
| | EsA | - | - | + |
| 5 | HbB | AA | BB | BB |
| | Cat | FF | SS | SS |
| | DIA I | SS | FF | FF |

breed or population which the animals belong to. As can be seen, the efficiency observed was low in the dominant systems but increased in the others, reaching the maximum value of 35-41% in Tf due to its high variability. Similar results were also obtained by STRATIL (1974) who found values ranging from .40 to .56 for Tf and .12 to .18 for HbB, with a mean exclusion probability of 57% in Czechoslovakian sheep breeds. If we compare the results of probabilities obtained in our data with those obtained using the same proteins and the gene frequencies described by MANWELL & BAKER (1977) for Poll Dorset and Australian Merino sheep, we verify similar results. This is a reflex of the good efficiency of these systems in pedigree verifications. Notwithstanding the possibility to predict an extensive use of the polymorphic systems studied here for pedigree controls is limited because the knowledge about allele frequencies in the various sheep breeds in Brazil is sparse.

Using the genealogic information about the animals of the Romney/Booroola flock, it was possible to identify 35 groups (sire,dam and lamb). Five parentage exclusions were made among them, corresponding to 14% of the cases (Table III). As can be seen, one of these was a mother exclusion that was probably due to an error in animal identification. In the other exclusions there was not a good agreement between the estimated and observed exclusion efficiency. However, the small number of cases studied does not allow to attribute greater significancy to this discrepancy. These data are similar to those found by HALL (1975) (16% of exclusions) and HOJNY & STRATIL (1978) (8-20% of false offspring). This result is however surprisingly high if we consider that we investigated two experimental controlled flocks carefully managed. In extensive breeding it is possible to found higher values which could therefore delay the improvement programmes.

It may be concluded that the systems investigated in this study can be useful for routine pedigree verification. The inclusion of other informative systems and blood groups, will probably increase the efficiency of parentage tests to values near the maximum (100%). It is important to point out that the knowledge of genetic variability also allows to estimate, in absence of exclusion, the relative chance of paternity (paternity index) in order to avoid incorrect pedigrees in data banks of breeding organizations. Private farmers could employed also this methodology to improve animal production.

Parentage tests in sheep have not been enforced in Brazil up to now, but the establishment of this technique is important for the prevention of non-paternity on excelent flock.

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