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Molecular techniques for the control of diseases in sheep. An application to control scrapie in sheep

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SUMMARY – The program aims to establish a nucleus unit with animals of the RR genotype from which to draw genetic material in terms of live animals and semen. Four hundred DNA samples from Chios sheep were screened for polymorphisms in the *PrP* gene using DGGE. A total of 101 RR and 202 RQ genotypes were detected and were used in private flocks and in the nucleus, breeding unit. The remaining ninety-seven individuals tested were found susceptible to scrapie and consequently they were slaughtered. The sex distribution of genotypes of the nucleus unit is 58 RR and 144 RQ females and 42 RR males.

Key words: Scrapie, DGGE, diseases, sheep, genotypes.

RESUME – *"Techniques moléculaires pour la lutte contre les maladies chez les ovines. Une application à la lutte contre le scrapie chez les ovines". Le programme vise à établir une unité de noyau avec des animaux du génotype RR afin de disposer de matériel génétique en termes d'animaux vivants et de sperme. Quatre cents échantillons d'ADN provenant des moutons de Chios ont été examinés pour des polymorphismes dans le gène* PrP *en utilisant DGGE. Un total de 101 RR et 202 génotypes RQ ont été détectés et ont été utilisés en troupeaux privés et en noyau, en unité d'amélioration. Les 97 individus examinés restants ont été trouvés susceptibles de scrapie et par conséquent ont été abattus. La distribution des sexes des génotypes de l'unité de noyau est 58 femelles RR et 144 femelles RQ et 42 mâles RR.*

Mots-clés : *Scrapie, DGGE, maladies, ovins, génotypes.*

Introduction

Scrapie is a fatal, degenerate disease affecting the central nervous system of sheep and goats. Scrapie has had a significant impact on the sheep industry and has caused severe financial losses to sheep producers. The disease is thought to be most commonly spread from ewe to offspring and to other lambs in contemporary lambing groups. Signs or effects do not usually appear until 2 to 5 years after the animal is infected (Cuille and Chelle, 1938).

Scrapie belongs to the same category of spongiform encephalopathy that affect the cow and humans (GSS and CJD). They are all heritable but also contagious. The causative agent is a polymorphic prion not fully characterized yet (Dickinson *et al.*, 1965). The clinical symptoms are well documented. Infection is associated with an aberrant form of a cell glycoprotein that triggers the *PrP* gene. The infectious form is triggered by the agent (the prion). Genetic variations among different breeds and the stain of scrapie may play a role in whether a sheep will contact the disease and how quickly clinical symptoms will appear. Clinical symptoms include scratching and rubbing against objects, loss of coordination, loss of weight, high stepping of the fore legs, swaying of the back and biting of feet and limbs.

Different allelomorphs have been identified so far from the expression of the codons 136, 154 and 171 in Cyprus, i.e. A136R154Q171, A136R154R171, V136R154Q171, A136H154Q171, A136R154H171, A136R154K171, T136R154K171. The allelomorph ARR has been associated with low susceptibility to the disease in all sheep breeds. With regard to natural scrapie, the 171 Arg allele (Goldmann *et al.*, 1990) does not appear to be associated with scrapie susceptibility.

DNA testing (genotyping) is definitely a tool. This fact will be utilized in a breeding program, which aims at the production and distribution of live animals and semen with the genotype that is considered not susceptible to scrapie. It is anticipated that by reducing the incidence of the disease and increasing the frequency of the desirable allele, and consequently genotypes, the disease can be controlled and/or eradicated.

Materials and methods

Animals

A small number of animals of both homozygous (RR) and heterozygous (RQ) genotypes were identified and transferred to a unit to establish a nucleus flock of Chios ewes. Matched matings are performed between animals with known genotypes and all progeny is subjected to genotype mapping following weaning. The female progeny from such matings is kept to replace QQ and RQ genotypes in the unit. It is anticipated that, by the end of 2003, only ewes of the RR genotype will constitute the nucleus population. A small proportion of the male progeny is kept for breeding purposes at the unit. All other males are either distributed to livestock keepers, whose flocks have been infected with the disease or they are transferred to the Artificial Insemination centre for semen collection. Hence, changes in gene and genotypic frequency in the general population will be achieved by the distribution of live animals and semen with RR genetic constitution. Semen collected at the Artificial Insemination centre is stored and used subsequently as requested following oestrus synchronization in units where the disease was identified.

All farms where the disease has been identified are under quarantine. All animals exhibiting clinical symptoms are slaughtered and carcasses are destroyed. The government of Cyprus has implemented an aid program through which farmers receive a compensation for each animal that is destroyed because of scrapie.

DNA amplification

DNA was purified from peripheral blood leukocytes using standard procedures. In sheep, as in other species, the *PrP* coding exon is uninterrupted (Goldmann *et al.*, 1990). Samples for Denaturing Gradient Gel Electrophoresis (DGGE) were amplified according to Saiki *et al.* (1988) in 100 µl reaction mixtures containing 0.5 to 1 µg of genomic DNA, 50 m*M* KCl, 10 m*M* Tris-HCl, pH 8.3, 1.5 m*M* $MgCl_2$, 0.01% gelatin, 200 µ*M* of each dNTP, 30 pmol of P2-P3 (Table 1) and 2.5 units of *Taq* polymerase (Pharmacia). The reactions were performed using a DNA thermocycler (Biometra) as follows: cycles of one minute denaturation at 94°C, one minute annealing at 59°C and two minutes elongation at 72°C were repeated 30 times with the last 72°C incubation extended to seven minutes.

Table 1. Amplification and sequencing of primers

Primers†	Position††	Sequence
P2	645-626	5′ CTGTGTGTTGCTTGACTGTG 3′
P3	217-236	5′ GCAACCGCTATCCACCTCAG 3′

†Primers P2, P3 used are according to Laplanche *et al.* (1993).
††Nucleotides are numbered according to Goldmann *et al.* (1990).

Denaturing gradient gel electrophoresis

DGGE was run according to the procedures described by Myers *et al.* (1987). Each PCR product (15 µl) was loaded on a 6.5% polyacrylamide gel containing a linearly increasing gradient from 20 to 80% (v/v) denaturant (100% denaturant = 7 *M* urea / 40% formamide (v/v) and electrophoresed over night at 160 V in a temperature-controlled bath heated to 60°C. Gels were stained with ethidium bromide and examined by UV transillumination.

Results and discussion

The primers were selected to provide a suitable fragment for DGGE analysis. As shown in Fig. 1,

DGGE of the *PrP* coding sequence PCR products from seven selected Chios sheep clearly revealed that the amplified fragments were polymorphic. Examination of the PCR products on a neutral polyacrylamide gel excluded the possibility that the shifted bands observed in the denaturing gel were due to allele size differences (data no shown). The most frequently observed electrophoretic patterns consisted of only one band (homozygous RR, Fig. 1, lanes 1, 2, 3, 5, 6) or four bands (Fig. 1, lanes 4, 7). In these last two cases, the two lower bands correspond to distinct homoduplexes and the two upper bands to the two heteroduplexes formed in heterozygous individuals between the two alleles by the reassorting of strands during PCR. Due to single base mismatches, heteroduplexes are destabilized and therefore melt earlier in the denaturing gradient gel and usually separate from each other. The nucleotide polymorphisms associated with each type of fragment mobility in the DGGE system (Fig. 1) were directly established by direct sequencing of PCR products.

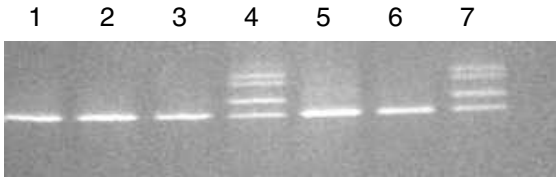

Fig. 1. Denaturing gradient gel pattern of the *PrP* coding sequence amplified fragments (P2-P3) from seven Chios sheep. Polymorphic codon genotypes are: 171 Arg/Arg (lanes 1, 2, 3, 5, 6) and 171 Arg/Gln (lanes 4, 7).

Following matched matings between known genotypes in the nucleus unit 400 Chios sheep DNA samples were screened for polymorphisms in the *PrP* gene with DGGE. A total of 101 RR and 202 RQ genotypes were detected. The remaining ninety-seven individuals tested were found to be susceptible to scrapie and consequently they were slaughtered. The genotype by sex distribution is presented in Table 2.

Table 2. Distribution of genotypes by sex

Sex	Genotypes	
	RR	RQ
Males	72	29
Females	58	144

All RQ as well as 30 RR male genotypes were distributed to private units with known incidence of scrapie. Fourteen RR males were transferred to the Artificial Insemination centre for semen collection and 14 more were provided to the Government breeding unit at Orites for genetic crosses. The remaining 14 RR male genotypes were kept at the nucleus unit for evaluation for other traits under the current breeding program. They will be used in individual matings and for the production of semen as needed. All females, regardless of genetic constitution (RR and RQ) are kept in the nucleus unit in order to replace the remaining QQ genotypes and expand the current flock population to the desired 500 ewes.

References

Cuille, L. and Chelle, P.L. (1938). La tremblant du mouton est bien inoculable. *C. R. Acad. Sci. Paris*, 206: 78-79.

Dickinson, A.G., Young, G.B., Stamp, J.T. and Renwick, C.C. (1965). An analysis of natural scrapie in Suffolk sheep. *Heredity*, 20: 485-503.

Goldmann, W., Hunter, N., Foster, J.D., Salbaum, J.M., Beyreuther, K. and Hope, J. (1990). Two alleles of a neural protein linked to scrapie in sheep. *Proc. Natl. Acad. Sci. USA*, 87: 2476-2480.

Laplanche, J.L., Chatelain, J., Westaway, D., Thomas, S., Dussaucy, M., Brugere-Picoux, J. and Launay, J.M. (1993). *PrP* polymorphisms associated with natural scrapie discovered by denaturing gradient gel electrophoresis. *Genomics*, 15: 30-37.

Myers, R.M., Maniatis, T. and Lerman, L.S. (1987). Detection and localization of single bases changes by denaturing gradient gel electrophoresis. In: *Methods of Enzymology*, Wu, R. (ed.). Academic Press, New York, pp. 501-527.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. and Elrich, H.A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, 239: 487-494.