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Analysis of PrP genotypes in relation to reproductive and production traits in Chios sheep

I.M. Ioannides *, A.P. Mavrogenis, C. Papachristoforou

Agricultural Research Institute, PO Box 22016, Nicosia, Cyprus

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ABSTRACT

The study describes the changes with time in gene and genotype frequencies of a closed Chios herd. Genomic DNA was isolated and purified from peripheral blood leucocytes using standard procedures. The identification of the allelic variants present in the DNA samples, was performed in a simple multiplex PCR reaction and melting curve analysis of the PrP gene. Only ARR/ARR female genotypes were kept for breeding, and only males of the same genotype were used following year 2 of the study. As a result of planned individual matings and selection, the susceptible ARQ/ARQ genotype was eliminated from the flock in 4 years. The gene frequency of the R allele from a low 0.056 at year 1 reached a high 0.911 at year 6 of the study. Data from first lactation ewes of known genotypes were used to examine possible associations between PrP genotypes and ewe reproductive (litter size at birth and at weaning) and production traits (litter weight at birth and at weaning and 60-day milk yield after weaning). No effects of the sire genotype (ARR/ARR and ARR/ARQ) were found for any of the traits studied. The ewe genotype was associated with performance for reproductive traits but not with total weight of lamb output at birth or at weaning. ARR/ARR ewes had a higher litter size at birth (2.09) compared to ARQ/ARQ ewes (1.79) and higher litter size at weaning (1.84) compared to ARR/ARQ ewes (1.59). 60-day milk yield after weaning was not influenced by genotype (114.8, 105.8 and 114.8 kg for ARR/ARR, ARR/ARQ and ARQ/ARQ genotypes, respectively). Birth weight and 98-day weight were not influenced by the lamb genotype. ARQ/ARQ lambs were slightly heavier ($P<0.05$) than ARR/ARR lambs at weaning as a result of faster pre-weaning growth. Post weaning growth was similar for the homozygous genotypes.

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1. Introduction

Scrapie is a fatal, neurodegenerative disease of sheep and goats that belongs to the family of transmissible spongiform encephalopathies (TSEs). Susceptibility to scrapie is associated with polymorphisms in ovine prion protein (PrP) gene on codons 136, 154 and 171. Using the single-letter code for aminoacids corresponding to these codons, the VRQ and ARQ allelic variants are associated with high susceptibility, while the ARR variant has the greatest resistance to the disease (Drögemüller et al., 2001). Infected flocks that contain a high percentage of susceptible animals can experience significant production losses. Scrapie has had a significant impact on the sheep industry and has caused severe financial losses to sheep producers. Animals sold from infected flocks spread scrapie to other flocks. 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).

The current European Union policy is towards the selection for resistant genotypes and eradication of genetically susceptible animals. Selection for ARR alleles could result in simultaneous changes in production traits (Brandsma et al., 2004; Alexander et al., 2005). In Cyprus, the first case of scrapie in sheep was diagnosed in 1985. Different allelomorphs (Ioannides et al., 2002) have been identified since the establishment of the scrapie breeding

* Corresponding author. Tel.: +357 22 403152; fax: +357 22 316770.
E-mail address: ioannide@arinet.ari.gov.cy (I.M. Ioannides).

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program in 1999. The allelomorph ARR has been associated with low susceptibility to the disease in all sheep breeds. With regard to natural scrapie, the 171 Arginine (R) allele (Goldmann et al., 1990) appears to be associated with resistance to scrapie.

The associations between the different genotypes with respect to PrP and ewe performance traits (fertility, litter size, ovulation rate, survival, etc.) have been studied by a number of investigators (Chase-Topping et al., 2005; Alexander et al., 2005; Vitezica et al., 2006). The effects of lamb genotype on lamb traits, such as birth weight, weaning weight, early gain and lean index have also been investigated (de Vries et al., 2004; Brandsma et al., 2004; Alexander et al., 2005). No studies have been conducted on the effects of different genotypes on milk production and total lamb output at weaning.

The aim of the program is to establish a nucleus unit with animals of the ARR/ARR genotype that is considered not susceptible to scrapie, from which to draw genetic material in terms of live animals and semen. It is anticipated that by reducing the incidence of the disease and increasing the frequency of the desirable allele, and consequently genotypes, the disease will be controlled and/or eradicated. The present work studies the association of PrP genotypes with reproductive and production traits of Chios sheep and describes the changes with time in gene and genotype frequencies in a nucleus population.

2. Materials and methods

2.1. Establishment of the population

The experimental Chios sheep unit of the Cyprus Agricultural Research Institute (ARI) in Athalassa, was used to establish a nucleus herd of resistant to scrapie genotypes (ARR/ARR). All the 289 animals of the initial population were genotyped and only one ewe was found to be of the ARR/ARR genotype. During the first year of the program, a Chios ram identified in a private herd to be ARR/ARR, was purchased and used, together with one ARR/ARQ and 9 ARQ/ARQ rams selected within the herd. The males used during the second year, included the purchased ram, along with 3 ARR/ARQ and 10 ARQ/ARQ rams selected within the herd. From year 3 onwards, only ARR/ARR rams selected within the herd were used. For breeding purposes, all rams were kept for one year and then sold to farmers, except for the purchased ram which was used for two consecutive years. Matched matings were performed between known genotypes and all progeny were subjected to genotype mapping at 42 (± 3) days of age, i.e. one week after weaning. The female progeny with ARR/ARR genotype were used to replace ARR/ARQ and ARQ/ARQ genotypes in the unit.

2.2. Data collection and statistical analysis

All animals were identified with ear tags and tattoo. The progeny from all matings were identified and weighed at birth. Sex and type of birth was also recorded. The female progeny kept for replacement, were utilized for the collection of production and reproductive characteristics of the three genotypes, i.e. ARR/ARR, ARR/ARQ and ARQ/ARQ. Litter size (number of lambs) and litter weight at birth and at weaning and the production of milk were recorded individually for each ewe. Data for lamb performance were collected for 660 individuals and concerned birth, weaning and 98-day weight, pre-weaning and post-weaning growth. Information on productive and reproductive traits was collected on 725 first lactation yearlings. Ewe productive and reproductive traits and lamb weights and gains were analysed by GLM procedures (SAS, 2006). The model for ewe traits included the fixed effects of the sire of lamb genotype (ARR/ARR or ARR/ARQ) and the fixed effects of the ewe genotype (ARR/ARR, ARR/ARQ or ARQ/ARQ). The model for the lamb traits included the fixed effect of lamb genotype only.

2.3. Denaturing Gradient Gel Electrophoresis (DGGE)

Genomic DNA was isolated and 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 DGGE were amplified according to Saiki et al. (1988) in 100 μl reaction mixtures containing 0.5 to 1 μg of genomic DNA, 50 mM KCl, 10 mM Tris–HCl, pH 8.3, 1.5 mM $MgCl_2$, 0.01% gelatin, 200 μM of each dNTP, 2.5 units of *Taq* polymerase (Pharmacia) and 30 pmol of each primer, P2 5′ CTGTGTGTTGCTTGACTGTG 3′ and P3 5′ GCAACCGCTATCCACCTCAG 3′ (Laplanche et al., 1993), which refer respectively to bp 645–626 and bp 217–236 of the PrP gene. The amplification reactions were performed in a Biometra thermocycler for 30 cycles of 1 min at 94 °C, 1 min at 59 °C and 7 min at 72 °C.

2.4. Genotype DGGE analysis

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

2.5. Real time PCR with melting curve analysis

Genomic DNA was isolated and purified from peripheral blood leukocytes using the High Pure PCR Template Preparation Kit (Roche Applied Science). Also DNA was isolated and purified using the MagNa Pure LC Ver. 3.0 automated DNA extraction instrument (Roche).

2.6. Genotype real time PCR with melting curve analysis

Genotype analysis was performed using the LightCycler 1.2 (three channels) and the LightCycler 2.0 (six channels), Real Time PCR systems (Roche). The most important allelic variants reported (VRQ, ARR, ARQ, ARH and AHQ) were checked using the LightCycler Scrapie Susceptibility Mutation Kit (TIB MOLBIOL, Germany). This dual colour LightCycler kit detects ovine prion protein gene mutations in the codons 136, 154 and 171. The TIB MOLBIOL kit was used in conjunction with the LightCycler FastStart DNA Master Hybridization Probes (Roche Applied Science). Performing this multiplex

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