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Characterization of the PRNP gene locus in Chios dairy sheep and its association with milk production and reproduction traits

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Summary

The objective of this study was to examine the prion protein gene locus (PRNP) in Chios sheep. PRNP is linked with scrapie resistance in small ruminants. Here, its impact on milk production (test-day and total lactation yield) and reproduction (age at first lambing, conception rate at first service, and prolificacy) was assessed. Genotyping at codons 136, 154 and 171 (classical scrapie) and 141 (atypical scrapie) was performed using DNA from milk somatic cells and PCR-RFLP analysis. A total of 1013 Chios ewes raised in 23 flocks were used. This constituted a random sample of the national breeding population. A total of 15 genotypes and 6 alleles linked to codons 136, 154 and 171 were detected. All animals were homozygous for the leucine allele at codon 141. Linear mixed models were used to assess the impact of PRNP genotypes and alleles on milk production and reproduction traits. The TRQ allele, whose association with such traits was assessed for the first time, had an adverse effect on age at first lambing. All other PRNP alleles, including ARR, which is associated with increased resistance to classical scrapie, had no significant effect on the traits studied. No significant associations of the PRNP genotypes with production and reproduction traits were observed. It was concluded that selection for scrapie-resistant sheep is not expected to affect the ongoing breeding programme that aims to enhance the milk yield and reproduction of the Chios breed.

Introduction

Scrapie is a transmissible spongiform encephalopathy (TSE) affecting sheep and goats. In sheep, classical scrapie appears to be controlled by genetic factors, with polymorphisms at codons 136 (A or V), 154 (Q or R) and 171 (Q, R or H) of the PRNP gene locus being the determining parameters (Hunter et al. 1996). The five most common alleles found in the literature (ARQ, AHQ, ARR, ARH and VRQ) may be combined in a total of 15 possible PRNP genotypes. Sheep homozygous for the ARR allele are resistant to natural and experimental classical scrapie, and bovine spongiform encephalopathy (BSE) infections, whereas animals carrying the VRQ allele are at the greatest risk of infection (Drogemuller et al. 2001). In breeds lacking the VRQ allele, ARQ homozygous animals are considered to be susceptible to scrapie infections (Hunter et al. 1997).

Recently, a previously undetected form of ovine prion disease, called atypical scrapie, with a different epidemiology and clinical and biochemical phenotype to classical scrapie was reported (Buschmann & Groschup 2005). PRNP genotypes are linked to atypical scrapie in a very different way compared to classical scrapie. A strong association of atypical scrapie occurrence with a leucine/phenylalanine (L/F) polymorphism at codon 141 of the PRNP gene locus was found in sheep with the ARQ allele in codons 136, 154 and 171 (Moum et al. 2005). Animals with the ARQ allele that also carry phenylalanine at codon 141 (AFRQ) are sensitive to atypical scrapie, whereas those carrying leucine (ALRQ) are resistant to the disease. Furthermore, the AHQ allele has also been associated with increased risk of atypical scrapie infection, while the ARR allele seems to give no protection to this form of the disease (Moum et al. 2005; Benestad et al. 2008).

The possibility that BSE may have entered the sheep population is a major concern, and this is important because BSE is not distinguishable from scrapie by clinical signs or current rapid tests. The latter has prompted the European Commission to require each member state to introduce breeding programmes to select for resistance to TSE in all sheep breeds (European Union, 2003). Breeding programmes to control classical scrapie have also been implemented by the US government (US Department of Agriculture 2001). These programmes aim to increase the frequency of the ARR allele and reduce the frequency of alleles known to contribute to TSE susceptibility (e.g. VRQ). If, however, the latter were favourably associated with economically important traits, then selection against them would compromise the success of the genetic improvement programme. Furthermore, if the favoured alleles were rare, then selection might increase inbreeding and reduce genetic variability (De Vries et al.

2004a). Selection for scrapie resistance must therefore be integrated into an overall genetic improvement scheme. However, before such a scheme is developed, it is important to determine the PRNP profile of each breed and assess the effect of the PRNP locus on other economically important traits.

In dairy sheep breeds, PRNP genotypes have been associated with milk yield, somatic cell count and fat and protein contents of milk. Population studies have been conducted for the Lacaune breed in France (Barillet et al. 2002), Churra in Spain (Á lvarez et al. 2006), East Friesian in Germany (De Vries et al. 2005), and Sarda (Salaris et al. 2007), Comisana (Pinelli et al. 2006) and Valle del Belice (Van Kaam et al. 2006) in Italy.

PRNP genotypes have also been associated with dairy ewe prolificacy in East Friesian (De Vries et al. 2005; Lipsky et al. 2006) and Rasa Aragonesa (Ponz et al. 2006) populations. Information about other traits related to reproductive performance is scarce, mainly because of relatively limited recording of such traits in both dairy and meat sheep breeds (Sweeney & Hanrahan 2008). In dairy sheep, the association of PRNP genotypes with age at first lambing and lambing intervals has been studied only in the East Friesian breed (De Vries et al. 2005; Lipsky et al. 2006).

Dairy sheep are of primary importance to the Greek livestock industry, with approximately 6.3 million milking ewes in the national population, rendering it the second largest national dairy flock in the European Union (de Rancourt et al. 2006). The Chios breed is the most prolific and highest milk-producing breed in Greece (Hatziminaoglou et al. 1996). A selection scheme was established in 1997 with the goal to improve milk production and reproduction. The presence of a rare PRNP polymorphism, threonine at codon 136 (p.Thr136), has been documented in this breed (Billinis et al. 2004), but there have been no population-wide studies of the genetic profile of the breed and the association of the PRNP locus with economically important traits. Thus, the objective of this study was to characterize the PRNP gene locus in Chios sheep for classical and atypical scrapie and to investigate its association with milk yield and reproduction traits.

Materials and Methods

Animals and measurements

The animal population used in this study comprised 1013 purebred ewes of the Chios dairy breed raised in 23 flocks that are members of the national Chios Sheep Breeder's Cooperative. This is the official organization that maintains the flock book of the breeding population. The total population size is approximately 20 000 animals raised in 66 flocks nationwide. Flocks are mostly linked via sire sharing and animal sales. Flocks and animals for this study were randomly selected; therefore, the sample is considered representative of the national purebred population.

Monthly test-day milk records were obtained from the database of the Cooperative. These records included the flock code, animal identification, number of lactations, lambing date, test date, number of test and test-day milk yield. The latter reflected the 24-h yield based on the evening and morning milking record, according to the official A4 method of the International Committee for Animal Recording (ICAR, 2008). From this data set, total lactation milk yield and duration of lactation were calculated according to ICAR (2008) rules. Edits required test-day milk yield to be between 0.15 and 5 kg, total lactation milk yield between 20 and 750 kg and duration of lactation between 60 and 270 days.

An additional data set obtained from the Cooperative included mating date, mating ram, type of service (individual or group), lambing date and number of lambs born per individual ewe. It should be noted that, because of the seasonality of reproduction in the breed, most lambings in the database were between November and March. From the second data set, the age at first lambing, conception rate at first service (i.e. whether a ewe conceived or not following the first service) and prolificacy were assessed. Descriptive statistics for the five traits studied are shown in Table 1.

Animal genotyping

Individual milk samples of 50 ml were collected from each ewe. Genomic DNA was extracted from milk somatic cells using a modified extraction method optimized for ovine milk based on the Nucleospin Blood kit (Macherey-Nagel, Duren, Germany) (Psifidi et al. 2010). Amino acid polymorphisms at codons 136 (A, T or V), 141 (L or F), 154 (R or H) and 171 (Q, R or H) were identified by restriction fragment length polymorphism (RFLP) analysis of two different polymerase chain reaction (PCR) products (258 base pairs and 259 base pairs) using the enzymes BspHI, BspDI, RsaI and MnLI (New England Biolabs; UK).

Based on the methodology developed by Lu¨hken et al. (2004), BspHI and BspDI were used to detect p.Val136, p.His154, p.Arg171 and p.His171. RsaI was used to detect p.Thr136, while MnLI was used for p.Leu141. The two PCR products were produced in separate reactions, both using the upstream primer, PrPov1: 5' GTCAAGGTGGTAGCCACA-3' developed by Psifidi et al. (2008), along with the Do1: 5'-TGCAC AAAGTTGTTCTGGTTACTATC-3' or the Do2: 5'-GCACAAA GTTGTTCTGGTTACTATAT-3' downstream primer, respectively, reported by Lu¨hken et al. (2004). PCR amplifications were performed in 20-ll reaction mixtures containing 1 ll genomic DNA, 200 lM each primer (PrPov1/Do1 or PrPov1/Do2), 200 lM deoxynucleoside triphosphates, 1.5 mM MgCl2, 1 U Platinum_ Taq DNA polymerase (Invitrogen, Breda, the Netherlands) and 1X PCR buffer (10x). The temperature cycling protocol on a TAKARA Thermal Cycler consisted of denaturation at 95 °C for 20 s, annealing at 54 °C for 30 s and extension at 72 °C for 15 s. The cycling was repeated 40 times. Each PCR was initiated with a 3-min denaturation at 95 °C and terminated with a 5-min extension at 72°C. All PCR and RFLP products were analysed by electrophoresis on 3% agarose gels, stained with ethidium bromide and visualized on a UV transilluminator. To verify genotyping results, 22 animal samples were also sequenced.

Statistical analysis

Genotypic and allelic frequencies were calculated by counting. The Hardy–Weinberg equilibrium state of the PRNP locus was examined using a chi-square test. The population would be considered to be in Hardy–Weinberg equilibrium if it failed the chi-square test at the 0.05 level.

The effect of the PRNP gene on total lactation milk yield, age at first lambing and prolificacy was assessed with the following linear mixed model (1); each trait was analysed separately:

$$Y_{ijklm} = \mu + Fi + Y_jB_z + L_lA + P + a_m + pe_m + e_{ijklm} \ (1)$$

where Y_{ijklm} is the dependent variable (total lactation milk yield, age at first lambing, prolificacy); μ is the overall population mean; Fi is the fixed effect of flock (i = 1–23); YjBz is the fixed effect of the interaction of year (j = 1–4) and month (z = 1–5, for the period November-March) of lambing; L_lA is the fixed effect of the interaction of lactation number (l= 1–3, for first to third or larger parity) by age at lambing (continuous variable); P is the fixed effect of PRNP gene locus as described later; a_m is the random genetic effect of the ewe

(including all known pedigree for a total of 13 473 animals); pe_m is the random across-lactation permanent environment effect of the ewe; and e_{ijklm} is the random residual effect.

The effect of the PRNP locus on the various traits was assessed in two ways. First, the individual allele effect was fitted as a regression on 0, 1 or 2, where the latter is the number of copies of the allele. For example, for VRQ, the PRNP gene locus effect in model 1 would be assigned the values 0, 1 and 2 for sheep with no VRQ copies (e.g. ARQ/ARQ), 1 VRQ copy (e.g. VRQ/ARQ) and 2 VRQ copies (VRQ/VRQ), respectively. In this case, model 1 assesses the allele substitution effect at this gene locus at the observed frequency of each allele. Secondly, the animal genotype was fitted as a fixed effect in model 1 instead of the allele effect.

In preliminary analyses, all fixed effects included in model 1 had a significant effect on the traits of interest. Furthermore, in the analysis of total lactation milk yield, duration of lactation and prolificacy were added as fixed effects in model 1. When age at first lambing was the trait analysed, the fixed effect of the interaction of lactation number by age at lambing was removed from model 1, together with the random across-lactation permanent environment effect.

Conception rate at first service (scored as 1 if the ewe conceived with the first service and 0 otherwise) was analysed with model 2:

$$Y_{ijklm} = \mu + Fi + Y_jB_z + V_lA + Q_n + P$$
$$+ a_m + pe_m + s_o + e_{ijklm} \qquad (2)$$

where Y_{ijklm} is the conception rate at first service; Y_jM_k is the fixed effect of the interaction of year (j = 1–4) and month (k = 1–5, for the period May–September) of mating; V_lA is the fixed effect of the interaction of mating period (l = 1 for virgin ewes, l = 2–3 for ewes in their first and second or greater lactation) by age at mating (continuous variable); Q_n is the fixed effect of mating type (individual or group); s_o is the random effect of service ram; and all other effects are as in model 1. Test-day milk yield was analysed with model 3:

$$Y_{ijklmnop} = \mu + Fi + Y_jB_k + L_lA + T_mC_n + L_lA + P + L_lDa_0$$
$$+ L_lD_{peo} + e_{ijklmnop} \qquad (3)$$

$Y_{ijklmnop}$ is the test-day milk yield record; T_mC_n is the fixed effect of the interaction of year (m= 1–4) and month (n = 1–9) of the monthly test; L_lD is the fixed effect of the interaction of

lactation number and days in milk (D), modelled by a second-order polynomial; L_lDa_o is the random effect of the interaction of lactation number, days in milk and genetic effect (a) of the ewe (including all known pedigrees), modelled by a second-order polynomial; L_lDpe_o is the random effect of the interaction of lactation number, days in milk and across-lactation permanent environmental (pe) effect of the ewe, modelled by a second-order polynomial; all other effects are as described in model 1. Orthogonal polynomials were used in model 3 to describe the time trajectory and account for covariances between repeated records of the same animal.

In a separate series of analyses, the effect of the PRNP gene on milk yield, prolificacy and conception rate at first service was assessed using only first lactation records. Models 1, 2 and 3 without the lactation and across-lactation permanent environment effects were used in this regard.

The effect of the PRNP gene locus on conception rate at first service was also assessed by fitting a non-linear logit function to model 2, which accounts for the binary (0/1) nature of the trait.

In all cases, the impact of the PRNP gene locus on the trait of interest assessed with models 1, 2 and 3 was the marginal locus effect adjusted for all other sources of systematic variation and the animal polygenic effect.

All statistical analyses were conducted using the ASREML software package (Gilmour et al. 2002).

In the aforementioned analyses, the null hypothesis was always that of no allele substitution or genotype effect on the trait of interest. Inevitably, multiple hypothesis tests were made because several analyses were performed. However, the analysed traits are genetically correlated with each other, so not all analyses constitute truly independent statistical tests. To account for multiple testing, we considered the analyses of each allele and genotype as distinct, independent hypothesis tests. Based on genotyping results, there were six such tests in the analysis of alleles and fifteen in that of genotypic effects. A Bonferroni correction, based on the Holm–Bonferroni method (Holm 1979), was implemented to adjust for multiple testing.

Results

Genotyping analysis

PRNP genotypic frequencies estimated for the 1013 Chios ewes are presented in Table 2. These results pertain to codons 136, 154 and 171. Fifteen genotypes (of a maximum possible of 21) were detected for the six alleles identified at codons 136, 154 and 171. The most frequent genotype was ARQ/ARQ (55.97%), followed by ARQ/AHQ (15.00%), ARQ/TRQ (11.94%) and ARQ/ARR (11.35%). The frequency of the remaining genotypes was close to or less than 1%. Allelic frequencies derived from genotypic frequencies were 76.10%, 8.20%, 7.70%, 6.90%, 0.70% and 0.40% for ARQ, AHQ, TRQ, ARR, ARH and VRQ, respectively. The PRNP locus was found to be in Hardy–Weinberg equilibrium ($P > 0.05$), as far as codons 136, 154 and 171 are concerned, suggesting that no direct or indirect selection has been exerted on this locus.

Regarding codon 141, which is linked to atypical scrapie, only the leucine polymorphism was detected in all samples.

Therefore, no further association study of this codon with traits of interest was conducted.

For the 22 animals that were also sequenced, genotypes were consistent across the two methods (sequencing and RFLP analysis).

Association analysis

Table 3 shows the effect of PRNP allele on milk production and reproduction traits when all lactation data were analysed.

These effects refer to substituting one allele for the average of all other alleles. For example, the substitution of one copy of ARQ for the average of all other alleles was associated with a reduction of lactation milk yield by 5.92 kg. Similarly, the same substitution was associated with a reduction of test-day milk by 0.0395 kg. Nevertheless, these particular effects were not significant ($P < 0.10$ and >0.05). However, the relatively low P-value, albeit not corrected for multiple testing at this stage, might still be suggestive of a potential association that was not supported statistically in this study, possibly because of sampling.

Significant ($P < 0.05$) PRNP allele substitution effects were found for TRQ on test-day milk yield and age at first lambing and for ARQ on conception rate at first service and age at first lambing (Table 3). Replacement of the average allele with a copy of TRQ would lead to an increase in testday milk by 0.0822 ± 0.037 kg (6% of the mean, $P = 0.027$) and an

unfavourable increase in the age at first lambing by 0.60 ± 0.20 months (4% of the mean, P = 0.003). Similarly, a copy of ARQ was associated with a decrease in age at first lambing by 0.30 ± 0.14 months (2% of the mean, P = 0.034) and an increase in conception rate by 3% (0.03 ± 0.01, P = 0.017). Following the Bonferroni correction, however, only the effect of TRQ on age at first lambing remained significant. Only the latter effect can be considered definitely significant, whereas the other three can be viewed as possible trends not supported by the Bonferroni test.

A few other effects with significance levels between 0.05 and 0.10 were also found (ARQ on lactation and test-day milk yield and AHQ on conception rate at first service). All remaining allele effects had a definitely non-significant (P > 0.10) association with milk production and reproduction traits across all lactations.

Non-statistically significant effects with low P-values (P < 0.10 and >0.05) were found for ARQ with first lactation test-day milk yield and for TRQ with first lambing prolificacy. All other first lactation results were definitely non-significant (P > 0.10).

Overall, genotype effects were not statistically significant (P > 0.10) for any trait. However, individual genotypes carrying alleles with a significant substitution effect were distinct, for example, genotypes carrying TRQ (e.g. TRQ/ VRQ, TRQ/ARQ) were associated with increased age at first lambing.

Analysis of the binary trait success of conception at first service with a non-linear model fitting a logit function did not yield results different from those described above.

Discussion

The aim of this study was to characterize the PRNP gene locus, which is responsible for classical and atypical scrapie, in the Chios dairy breed of sheep and to examine its effect on milk production and reproduction traits. In recent years, selection programmes aiming at increasing the frequency of genotypes resistant to classical scrapie (e.g. ARR/ARR) have been implemented throughout Europe. However, the association of the PRNP locus with important production and reproduction traits would determine whether selection against scrapie might also affect breeding programmes aiming at genetically improving these traits.

Genotyping analysis

Allelic frequencies at the PRNP locus estimated in the present study which are linked to classical scrapie (codons 136, 154, 171) were somewhat different from allelic frequencies

reported for Chios sheep in two previous experimental studies. The present genotyping results showed that only 0.39% of the Chios sheep population belong to the most resistant to scrapie genotype (ARR/ARR), whereas 12.9% are heterozygous carriers of ARR. Ekateriniadou et al. (2007a) genotyped 65 Chios sheep from mainland Greece and found that 10.8% had the ARR/ARR genotype, whereas 26.2% were heterozygous ARR. Billinis et al. (2004) genotyped 110 Chios sheep and found 14.5% heterozygous ARR animals and no animals homozygous for this allele. It should be noted that data sizes differed considerably in these studies; the random sample of 1,013 ewes drawn for the present study is viewed as representative of the population as a whole.

The most common genetic variant in the present study was the so-called wild-type allele (ARQ) with a frequency of 76.1%. Ekateriniadou et al. (2007a) found this allele at a frequency of 50.8% and Billinis et al. (2004) at a frequency of 80%. The frequency of the most susceptible allele (VRQ) was very small (0.4%) in the present study, while it was not detected at all in the studies by Billinis et al. (2004) and Ekateriniadou et al. (2007a).

In the light of these results, Chios may be considered one of the so-called alanine breeds, characterized by a high prevalence of the wild-type ARQ and a limited frequency of VRQ (Baylis & Goldmann 2004). The proportions of AHQ (8.8%) and TRQ (7.7%) found in the present study were moderate. In Chios sheep, the AHQ allele seems to favour classical scrapie infection (Ekateriniadou et al. 2007b). In some other breeds, such as the German Merinoland sheep (Lu¨hken et al. 2004), the AHQ allele is associated with high susceptibility to scrapie, whereas in other populations such as the Cheviot sheep, this allele is associated with increased resistance to scrapie and long incubation periods (Hunter et al. 1996).

TRQ is a new allele, recently detected in Chios sheep in Greece (Billinis et al. 2004) and in native breeds in Cyprus and Turkey (Un et al. 2008). In the present study, TRQ was detected for the first time with RFLP using enzyme RsaI; in the previous studies, denaturing gradient gel electrophoresis and direct sequencing were used for this purpose. The TRQ allele has not yet been formally associated with scrapie, but it is thought not to affect susceptibility of the Chios breed (Billinis et al. 2004). However, owing to the limited scale of that study, further research is required to determine the exact association between TRQ and susceptibility to scrapie.

Regarding polymorphisms associated with atypical scrapie (codon 141), all animals in the present study were homozygous for the leucine allele, which is linked to resistance to this form of the disease. No previous reports regarding Chios sheep are available in this regard. Indeed, there is still very limited knowledge in the international literature about these alleles,

because they are not detected in routine genotyping for the selection programmes of ovine breeds. Only one recently published study of five Italian breeds gave insights into their susceptibility to atypical scrapie (Pongolini et al. 2009). AFRQ was present at a frequency of 2.53% in the Sarda dairy breed, while it was absent in the Comisana dairy breed. The frequency of this allele in the three meat breeds of this study (Bergamasca, Appenninica, Massese) ranged from 0.34% to 10.70%. Genotyping at codon 141 in the Pongolini et al. (2009) study was performed using a multiplex primer extension assay, whereas in the present study it was carried out by RFLP analysis using the MnLI restriction enzyme. Because codon 141 was found to be monomorphic in the present study, no associations with traits of interest were found. All discussion from this point onwards relates to association studies of alleles and genotypes at codons 136, 154 and 171, which are mainly linked to classical scrapie.

Association analysis

For the most part, published association studies have estimated, by virtue of their design, the effect of different PRNP genotypic classes on various traits and thus have been less able to detect the effect of individual PRNP alleles. In the present study, to evaluate the effect of allele substitution on test-day and total lactation milk yield for all PRNP alleles, genetic effects were defined by the number of allele copies carried by each genotype. Only Pinelli et al. (2006) have assessed the effect of specific alleles on milk yield of the Comisana dairy sheep using similar methods. In their study, only two alleles (ARQ and VRQ) were investigated, and their effect on milk yield was negative but not significantly different from zero. The results of the present study also suggest a negative but not statistically significant effect of ARQ on test-day and total lactation milk yield ($P = 0.086$ and 0.065, respectively). The positive significant ($P = 0.027$) effect of the TRQ allele on test-day milk yield found in the present study is the first report of this result.

The effect of the PRNP genotypes on test-day and total lactation milk yield was also analysed. Differences found between the genotypes of Chios sheep were negligible and not significant. These results are consistent with previous studies of other dairy breeds such as Lacaune (Barillet et al. 2002), East Friesian (De Vries et al. 2005), Churra (Alvarez et al. 2006), Sarda (Salaris et al. 2007) and Comisana (Pinelli et al. 2006). In contrast, Van Kaam et al. (2006) found a strong association between PRNP polymorphisms at codon 154 and milk yield in Valle del Belice dairy ewes, with polymorphism His at this codon having a

favourable effect. However, this result was mainly based on only one AHQ/AHQ animal, leading the authors to recommend caution in interpretation of the data.

The effect of PRNP alleles and genotypes on prolificacy of Chios sheep was not significantly different from zero in the present study. This result is generally consistent with previous reports in the East Friesian dairy breed (De Vries et al. 2005; Lipsky et al. 2006) and meat breeds such as Beclare (Sweeney et al. 2007), Columbia (Alexander et al. 2005), Hampshire (Alexander et al. 2005), Suffolk (De Vries et al. 2004b; Lipsky et al. 2006) and Texel (De Vries et al. 2004b). However, our results did not support the conclusions of a recently published study based on data from an experimental station of Chios sheep in Cyprus, where ARR/ARR and ARR/ARQ genotypes were found to increase prolificacy compared to the ARQ/ARQ genotype (Ioannides et al. 2009). However, there are considerable differences in the methodologies and the animal populations used in the two studies that may explain the different results obtained. Ioannides et al. (2009) studied an experimental flock of 725 ewes in Cyprus that had been used to establish a nucleus of animals resistant to scrapie (only genotypes carrying ARR and ARQ were included), whereas the present study was based on a large sample of 1,013 randomly selected ewes from the general population. In fact, animals used in the present study were from nearly half of all purebred Chios flocks that are members of the national Chios Sheep Breeders Cooperative in Greece. Furthermore, in the present study, results were adjusted for other factors affecting prolificacy, and a multiple testing correction was implemented.

Studies regarding the effect of PRNP alleles and genotypes on age at first lambing are largely missing from the international literature. The results of the present study indicate that there is no association between PRNP genotypes and age at first lambing, in agreement with previous studies of the East Friesian dairy sheep (De Vries et al. 2005; Lipsky et al. 2006) and the Suffolk and German Black-Headed meat breeds (De Vries et al. 2004b; Lipsky et al. 2006). However, in the present study, the TRQ allele had a significant adverse effect on the trait, suggesting that carriers of this allele tend to mature later. On the other hand, the ARQ allele was found to have a significant favourable effect on the trait, which is associated with earlier first lambing. This result, combined with the high frequency of the ARQ allele in the population, is consistent with the early maturity that is a feature of the Chios breed (Hatziminaoglou et al. 1996). No previous studies on the association between TRQ and age at first lambing have been reported in the literature; in fact, TRQ has never been studied before in association with any trait in sheep of breeds where this allele has been found.

There is very little published literature on the direct association of the PRNP gene locus with conception rate at first service in either dairy or meat sheep (Sweeney & Hanrahan 2008). There is no study based on population data, and the only report found is that of Casellas et al. (2007), who used an experimental flock of Ripolessa sheep, an autochthonous meat breed in Spain. In the study of Casellas et al. (2007), no association between PRNP alleles or genotypes with conception rate was found. In the present study, ARQ was associated with somewhat increased conception (although the effect did not maintain significance status following the Bonferroni correction). Differences between the animal populations used in the two studies are acknowledged, especially the fact that Casellas et al. (2007) studied a meat sheep population raised as an experimental flock, whereas the present study was of a dairy sheep breed raised commercially.

In the present study, the ARR allele, which in homozygous form is associated with increased resistance to classical scrapie, did not have any effect on the traits investigated. The practical implication of this result is that selection to increase the frequency of ARR, within the scope of enhancing the breeds resistance to scrapie, is not expected to interfere with the conventional breeding programme aiming at improving milk production and reproduction. These results were in accordance with other dairy breed studies, which did not find any effect of ARR allele on milk production traits. Moreover, no evidence for an association between ARR allele and ovulation rate or fertility was found in an experimental study of crosses between the meat sheep breeds Berrichon du Cher and Romanov (Vitezica et al. 2006). In addition, no evidence of an additive effect of the ARR allele on age at first lambing has been found in other studies (De Vries et al. 2004b). However, a significant

positive effect of the ARR allele on the interval between second and third lambings was revealed in German Blackhead Mutton sheep (meat breed), whereas an adverse effect of ARR on this trait was found in Suffolk meat sheep (De Vries et al. 2004b). Moreover, previous studies found no association between the ARR allele and prolificacy (Sweeney & Hanrahan 2008), except for a positive effect found in Texel (Brandsma et al. 2004) and a negative effect in Whiteface Western (Alexander et al. 2005) and Suffolk meat sheep (Alexander et al. 2005). Nevertheless, the evidence of an ARR effect on prolificacy in Suffolk and Texel sheep was inconsistent across studies and was generally weak (Sweeney & Hanrahan 2008).

The present study investigated the association between both PRNP alleles and genotypes with various traits of interest. Although both may describe the overall genetic effect of the PRNP locus, the former mainly describes the additive effect of each allele, whereas the latter may account for non-additive (dominance) interactions between alleles. The aim of most scrapie

selection programmes, however, is to increase the frequency of the ARR allele and reduce the frequency of undesirable alleles (e.g. VRQ). Therefore, association studies of both PRNP alleles and genotypes may be more conclusive than studies of genotype only. Moreover, significant effects of PRNP alleles were found in the Chios breed for certain reproduction traits, suggesting that the potential impact of the PRNP locus on reproduction should be investigated together with production, before selection for scrapie resistance is implemented in a sheep population. The PRNP gene may be in different phases of linkage disequilibrium with genes affecting reproductive performance in different populations, meaning that separate association studies for each breed are warranted.

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Legends

Tab.1 - Descriptive statistics for milk yield and reproduction traits of Chios sheep.

Tab.2 - Genotypic frequencies at the PRNP locus of Chios sheep

Tab.3 - PRNP allele substitution effect (h, standard error in parentheses) on milk production and reproduction traits across all lactations.

Tab.1

Trait	Records (n)	Mean	SD
Total lactation milk yield (kg)[1]	1839	217.2	106.9
Test-day milk yield (kg)	8235	1.40	0.77
Prolificacy (no. lambs)	2027	1.81	
Success of conception at first service (0/1)[2]	2226	0.83	
Age at 1st lambing (mo)	838	15.3	3.4

[1] Average duration of lactation 197.3 days.

[2] 1 if a ewe conceived at first service, 0 otherwise.

Tab.2

PRNP genotypes (codons 136, 154, 171)	N. ewes	Frequency (%)
ARQ/ARQ	567	55.97
ARQ/AHQ	152	15.00
ARQ/TRQ	121	11.94
ARQ/ARR	115	11.35
ARQ/ARH	14	1.38
TRQ/ARR	11	1.09
TRQ/TRQ	9	0.88
AHQ/ARR	5	0.50
ARQ/VRQ	5	0.50
AHQ/TRQ	5	0.50
ARR/ARR	4	0.39
AHQ/VRQ	2	0.20
AHQ/AHQ	1	0.10
TRQ/VRQ	1	0.10
AHQ/ARH	1	0.10
Total	1013	100.00

Tab.3

Trait	Allele (codons 136, 154, 171	H (SE)	P-value
Total lactation milk yield (kg)	ARQ	- 5.92 (3.21)	0.065
	AHQ	0.94 (4.88)	0.847
	VRQ	9.34 (21.68)	0.667
	TRQ	6.92 (5.19)	0.183
	ARR	6.86 (5.01)	0.172
	ARH	- 3.40 (15.22)	0.823
Test-day milk yield (kg)	ARQ	- 0.039 (0.023)	0.086
	AHQ	- 0.030 (0.034)	0.388
	VRQ	0.032 (0.163)	0.843
	TRQ	0.082 (0.037)	0.027[1]
	ARR	0.050 (0.036)	0.174
	ARH	0.012 (0.102)	0.904
Prolificacy (no.lambs)	ARQ	0.03 (0.03)	0.323
	AHQ	- 0.04 (0.04)	0.390
	VRQ	- 0.13 (0.20)	0.520
	TRQ	- 0.03 (0.05)	0.583
	ARR	- 0.01 (0.05)	0.797
	ARH	0.11 (0.14)	0.431
Success of conception at first service (0/1) *	ARQ	0.03 (0.01)	0.017[1]
	AHQ	- 0.03 (0.02)	0.091
	VRQ	- 0.01 (0.10)	0.902
	TRQ	- 0.01 (0.02)	0.537
	ARR	- 0.03 (0.02)	0.175
	ARH	0.00 (0.06)	0.939
Age at first lambing (mo)	ARQ	- 0.030 (0.014)	0.034[1]
	AHQ	0.04 (0.22)	0.856
	VRQ	- 0.63 (0.86)	0.464
	TRQ	0.60 (0.20)	0.003[1,2]
	ARR	0.17 (0.23)	0.460
	ARH	0.28 (0.65)	0.667

[1] significant (P < 0.05) prior to a Bonferroni correction.

[2] significant after a Bonferroni correction.

*1 if a ewe conceived at first service, 0 otherwise.