Development of a SNP parentage assignment panel in some North-Eastern Spanish meat sheep breeds

Jorge H. Calvo (Calvo, JH)1,2, Magdalena Serrano (Serrano, M)1, Flavie Tortereau (Tortereau, F)4, Pilar Sarto (Sarto, P)2, Laura P. Iguaucel (Iguaucel, LP)2, María A. Jiménez (Jiménez, MA)1, José Folch (Folch, J)2, José L. Alabart (Alabart, JL)2, Stéphane Fabre (Fabre, S)4, and Belén Lahoz (Lahoz, B)2

1 ARAID, Av. de Ranillas 1-D, 50018 Zaragoza, Spain. 2 CITA-IA2, Producción y Sanidad animal, Av. Montañana 930, 50059 Zaragoza, Spain. 3 INIA, Mejora Genética Animal, Ctra. La Coruña km 7.5, 28040 Madrid, Spain. 4 GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France.

Abstract

Aim of study: To validate two existing single nucleotide polymorphism (SNP) panels for parentage assignment in sheep, and develop a cost effective genotyping system to use in some North-Eastern Spanish meat sheep populations for accurate pedigree assignment.

Area of study: Spain

Material and methods: Nine sheep breeds were sampled: Rasa Aragonesa (n=38), Navarra (n=39), Ansotana (n=41), Xisqueta (n=41), Churra Tensina (n=38), Maellana (39), Roya Bilbilitana (n=24), Ojinegra (n=36) and Cartera (n=39), and these animals were genotyped with the Illumina OvineSNP50 BeadChip array. Genotypes were extracted from the sets of 249 SNPs and 163 SNPs for parentage assignment designed in France and North America, respectively. Validation of a selected cost-effective genotyping panel of 158 SNPs from the French panel were performed by Kompetitive allele specific PCR (KASP). Additionally, some functional SNPs (n=15) were also genotyped.

Main Results: The set of 249 SNPs for parentage assignment showed better diversity, probability of identity, and exclusion probabilities than the set of 163 SNPs. The average minor allele frequency for the set of 249, 163 and 158 SNPs were 0.41 + 0.01, 0.39 + 0.01 and 0.42 + 0.01, respectively. The parentage assignment rate was highly dependent to the percentage of putative sires genotyped.

Research highlights: The described method is a cost-effective genotyping system combining the genotyping of SNPs for the parentage assignment with some functional SNPs, which was successfully used in some Spanish meat sheep breeds.

Additional key words: autochthonous breeds; pedigree; genotyping; animal breeding

Abbreviations used: AI (artificial insemination); EBV (estimated breeding value); ISAG (International Society for Animal Genetics); KASP (kompetitive allele specific PCR); LOD (logarithm of the odds); MAF (minor allele frequency); PE (exclusion probability); PI (probability of identity); SNP (single nucleotide polymorphism)

Authors’ contributions: Conceived the research and designed the experiments: JHC, MS, SF, FT, BL; performed the experiments: PS, LPI; collected and codified the data: JHC, MS, JLA, MAJ, PS, LPI; analyzed the data: JHC, MS, MAJ; discussion and writing the manuscript: JHC, MS, FT, JF, JLA, SF, BL. The manuscript was proofread by all the co-authors.

Citation: Calvo, JH; Serrano, M; Tortereau, F; Sarto, P; Iguacel, LP; Jiménez, MA; Folch, J; Alabart, JL; Fabre, S; Lahoz, B (2020). Development of a SNP parentage assignment panel in some North-Eastern Spanish meat sheep breeds. Spanish Journal of Agricultural Research, Volume 18, Issue 4, e0406. https://doi.org/10.5424/sjar/2020184-16805

Supplementary material: (Tables S1, S2, S3 and S4) accompanies the paper on SJAR’s website.

Received: 08 Apr 2020. Accepted: 27 Oct 2020.

Copyright © 2020 INIA. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC-by 4.0) License.

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Jorge H. Calvo: jhcalvo@cita-aragon.es

Funding agencies/institutions | Project / Grant
--- | ---
FEDER within the POCTEFA framework | PIRINNOV1 EFA103/15
Platea I + D Aragón Government | FITE 2017
Research Group Funds of Aragón Government | A14_17R

Introduction

Breeding programs have the purpose to get sustainable genetic gains in one or several traits while controlling the loss of genetic variation. Traditional pedigree-based-BLUP (Best linear unbiased prediction) selection (Henderson, 1984) is used to calculate estimated breeding values (EBVs) obtained from performance records and pedigree information. However, the success of genetic evaluations systems is directly affected by the accuracy
of pedigrees. Complete pedigree information is a prerequisite to get accurate EBVs, correctly rank parents and offspring and maximize the genetic gain (Israel & Weller, 2000; Raoul et al., 2016). In this sense, the proportion of known sires is very low in Spanish meat sheep populations because the management (extensive or semi-extensive farming) relies very little on artificial insemination (AI) or natural mating with a single ram per group of ewes. Moreover, a number of these populations are considered as endangered breeds with reduced effective population size, and reared in small-sized flocks. Therefore, the implementation of a mating scheme based in pedigree information can control the inbreeding that is greatly affected by population structure (Gutiérrez et al., 2008).

In this situation, the number of ewes belonging to a breeding program nucleus remains limited, because only some of them are inseminated by or mated to a single identified ram. Furthermore, another source of incorrect pedigree record information is usually due to ewes failing to keep their litter together, or lamb desertion, that may lead to limit the selection response (Barnett et al., 1999; Visscher et al., 2002).

Therefore, genomic information like DNA markers can contribute to reconstruct the phylogenetic relationships of populations. Microsatellite markers have been used extensively for parentage control in sheep (Arruga et al., 2001; Glowatzki-Mullis et al., 2007; Saberivand et al., 2011; Visser et al., 2011; Souza et al., 2012; da Silva et al., 2014) and are recommended by the International Society for Animal Genetics (ISAG) as they are highly abundant and informative, relatively inexpensive to use, and generate satisfactory results in tests for paternity exclusion. However, as DNA markers in genomic selection studies (Meeuwissen et al., 2013), single nucleotide polymorphisms (SNPs) are now largely developed on SNPs chip arrays allowing high throughput genotyping (Heaton et al., 2002; Werner et al., 2004; Hayes, 2011). Recently, various SNP panels have been developed for sheep of different international breeds specifically for parentage assignment (Bell et al., 2013; Clarke et al., 2014; Heaton et al., 2014; Tortereau et al., 2017). The SNP panels developed from French breeds was the first panel based on European sheep breeds (Tortereau et al., 2017). These authors pointed out that four Spanish breeds (Churra, Ojala, Castellana and Rasa aragonesa) belonging to the Sheep HapMap breeds of the International Sheep Genomics Consortium (Kijas et al., 2012a) had similar minor allele frequency (MAF) values for the selected SNPs to that described in the French breeds, suggesting that this panel should perform well in these Spanish breeds.

In addition, in Spain, a national breeding program for resistance to classical scrapie was implemented. In the breeding programs, animals are genotyped, and those carrying favorable Prnp alleles for resistance are used as breeding animals (Hunter et al., 1997; Acín et al., 2004). In the same way, a selection program for prolificacy in Rasa Aragonesa breed implements the genotyping of reproducers for alleles associated to prolificacy (Calvo et al., 2020), as well as that related to reproductive seasonality (Calvo et al., 2018). Apart from these SNPs, some other SNPs are of interest to genotype for validation of their effects in these Spanish breeds, such as atypical scrapie susceptibility (Moum et al., 2005), lentivirus susceptibility infection (Heaton et al., 2012; Sider et al., 2013), or other alleles found in other breeds and related to prolificacy (Bodin et al., 2007; Drouilhet et al., 2013).

The objective of this study was to validate two existing SNP panels for parentage assignment in sheep, including the French panel, and develop a cost-effective genotyping system of a reduced set of SNPs in an open platform to use in some North-Eastern Spanish meat sheep populations for accurate pedigree assignment. In a second stage, we tested and validated the performance of the cost-effective genotyping system together with some functional SNPs in replacements lambs from different farms and breeds.

**Material and methods**

**Samples and genotyping**

**OvineSNP50 BeadChip array genotyping**

Three hundred and thirty-five ewes from nine Spanish sheep breeds were sampled: Rasa Aragonesa (n=38), Navarra (n=39), Ansoñana (n=41), Xisqueta (n=41), Churra Tensina (n=38), Maellana (n=39), Roya Bilibitana (n=24), Ojinegra (n=36) and Cartera (n=39). Sheep breeds considered in the current work are specialized in meat production. The Churra Tensina, Ansoñana, Maellana, Roya Bilibitana, Xisqueta and Cartera are considered endangered sheep breeds, having the first four a very low census ranging between 8,000 and 13,000 heads (https://www.mapa.gob.es/es/ganaderia/temas/zootecnia/razas-ganaderas/razas/catalogo/). Animals were selected as unrelated as possible. In this sense, the maximum number of animals selected from each flock was 4, and they were unrelated based on their pedigree records. Furthermore, to check Mendelian inheritance 11 duos (2, 1, 2, 2, 2 and 2 from Rasa aragonesa, Navarra, Ansoñana, Xisqueta, Churra Tensina, and Cartera, respectively) and 3 trios (2 and 1 from Navarra and Rasa Aragonesa sheep breeds, respectively) were also selected.

Genomic DNA was extracted from blood samples of the 335 ewes using the FlavorPrep Genomic DNA mini kit (Flavorgen, Ibian, Zaragoza, Spain). DNA samples were genotyped with the Illumina (San Diego, California, USA) OvineSNP50 BeadChip array designed by the International Sheep Genome Consortium (Kijas et al., 2020).
SNP parentage testing in some North-Eastern Spanish meat sheep breeds

2012b), SNP genotyping services were provided by the "Xenetica Fontao" company (www.xeneticafontao.com).

Validation by kompetitive allele specific PCR (KASP)
A total of 2,018 replacement ewe lambs from 12 farms and 3 breeds, and their putative fathers were sampled (428 sires). Only the candidate parents and their offspring were genotyped to perform paternity assignment. Furthermore, the 11 duos, the 3 trios, and 5 randomly selected samples from each breed (45 samples in total) were also genotyped to check the consistency between both genotyping approaches: OvineSNP50 BeadChip array and KASP (design and assays were performed by the LGC company, Biotools, Madrid, Spain). Validation of a selected cost-effective genotyping panel of SNPs from the French panel was performed by KASP. Additionally, some functional SNPs (n=15) were also genotyped located in BMP15 (n=3; Bodin et al., 2012; Sider et al., 2013), MTNR1A (n=1; Calvo et al., 2018), PRNP (n=7; Hunter et al., 1997; Acín et al., 2004; Moum et al., 2005), TMEM154 (n=2; Heaton et al., 2012; Sider et al., 2013) and HSP90AA1 (n=1; Salces-Ortiz et al., 2015). The functional SNP coordinates and affected traits are indicated in the Table 1. Results were visualized with SNP Viewer 2 software version 4.0 (LGC, 2013). Genotype data for each animal were exported for the statistical analysis.

Analysis of genotypic data
Selection of the SNP panel for parentage assignment
Firstly, we applied the quality control (QC) criteria on the raw genotypes obtained from the OvineSNP50 BeadChip array using PLINK 1.9 (Chang et al., 2015) as follows: i) Individuals with low call rate (< 0.97) were excluded from additional analysis; ii) SNPs with unknown location of the marker in the ovine chromosomes were excluded; iii) SNPs were also excluded if they showed a low call rate (< 0.97), a MAF < 0.05, or significant deviations from Hardy-Weinberg equilibrium (HWE) (p-value < 0.001) within breed. The subsequent analysis focused on two sets of SNPs; a first set of 249 SNPs published from the French panel for parentage assignment (Tortereau et al., 2017) and the 163 SNPs panel described in Heaton et al. (2014) used in the North American and globally diverse breeds. Paternity assignment effectiveness does not only depend on the number of SNPs used but also on the level of informativeness.

Table 1. Functional SNPs jointly genotyped with the subset of 158 SNPs from the French panel for the validation by Kompetitive allele specific PCR (KASP). Information about the location, dbSNP name in Ensembl variation database, gene, and associated phenotype. References associated to these genes and phenotypes are indicated by using a superscript.

| CHR | SNP position in Oar3.1 and allele variation | dbSNP name | Gene | Phenotype |
|-----|------------------------------------------|------------|------|-----------|
| 11  | 11:g.36638222G>A                         | rs58866278 | B4GALNT2 | Prolificacy/FecLL [1] |
| 13  | 13:g.46225659G>A                         | rs601660229| PRNP  | Classical scrapie susceptibility [2,3] |
| 13  | 13:g.46225660T>G                         | rs591379086| PRNP  | Classical scrapie susceptibility [2,3] |
| 13  | 13:g.46225674C>T                         | rs598580733| PRNP  | Atypical scrapie susceptibility [4] |
| 13  | 13:g.46225714G>A                         | rs605048948| PRNP  | Classical scrapie susceptibility [2,3] |
| 13  | 13:g.46225764A>C                         | NA         | PRNP  | Classical scrapie susceptibility [2,3] |
| 13  | 13:g.46225765G>A                         | rs160575103| PRNP  | Classical scrapie susceptibility [2,3] |
| 13  | 13:g.46225766G>T                         | rs400844237| PRNP  | Classical scrapie susceptibility [2,3] |
| 17  | 17:g.4857244G>A                         | rs408593969| TMEM154| Lentinususceptibility [5,6] |
| 17  | 17:g.4857350T>A                         | rs427737740| TMEM154| Lentinususceptibility [5,6] |
| 19  | 19:g.65645462C>G                         | rs397514116| HSP90AA1| Sperm DNA fragmentation [7] |
| 26  | 26:g.15118464C>G                         | rs403212791| MTNR1A| Reproductive seasonality [8] |
| X   | X:g.50971644-50971660indel              | rs421419167| BMP15 | Prolificacy/FecXR [9] |
| X   | X:g.50971170C>T                          | NA         | BMP15 | Prolificacy/FecXGR [10] |
| X   | 50971158C>T                              | NA         | BMP15 | Prolificacy/FecXL [11] |

CHR: chromosome. NA: not available. [1]Drouilhet et al., 2013; [2]Hunter et al., 1997; [3]Acín et al., 2004; [4]Moum et al., 2005; [5]Heaton et al., 2012; [6]Sider et al., 2013; [7]Salces-Ortiz et al., 2015; [8]Calvo et al., 2018; [9]Martinez-Royo et al., 2008; [10]Demars et al., 2013; [11]Bodin et al., 2007.
that these markers provide. To study the informativeness of the SNPs included in this work, three informative indexes were calculated for both sets of SNPs and for each population included in this study: the MAF, the exclusion probability (PE), and the probability of identity (PI) (Schütz & Brenig, 2015; Tortereau et al., 2017). PE is the probability to exclude one (PE1) or two (PE2) randomly sampled parent(s) from the parentage of an individual which is truly unrelated to them. PE1 assumes that genotypes are known for the offspring and a putative parent, but genotypes are not available for a known parent (one parent missing). PE2 assumes genotypes are known for the offspring, one confirmed parent, and one putative parent (both parents genotyped). PI is the probability that two randomly selected individuals in a population have identical genotypes for all the SNPs genotyped.

A reduced panel of 158 SNPs from the French panel was chosen to use in an open platform for a cost-effective genotyping for parentage assignment. Only SNPs with a MAF >0.3 and a call rate >0.97 in the 9 breeds were selected.

A reduced panel of 158 SNPs from the French panel was chosen to use in an open platform for a cost-effective genotyping for parentage assignment. Only SNPs with a MAF >0.3 and a call rate >0.97 in the 9 breeds were selected.

**Parentage assignment validation**

We carried out the paternity assignment in each of the ten farms by using the CERVUS software (Kalinowski et al., 2007). CERVUS uses a simulation procedure to determine the distribution of the critical values of logarithm of the odds (LOD) or Delta score for 80% and 95% confidence levels for the candidate father–offspring pairs. LOD score was used for paternity assignment. The simulation parameters were as follows: 10,000 simulated offspring, the number of candidate parents and the sampled sires was provided by the breeders’ association (varying between 50% and 100%), at least 90% loci having allele calls, with an estimated 5% genotyping error rate. We allowed one SNP genotype mismatch between offspring and its assigned sire because of technical genotyping failures.

**Results and discussion**

We selected two sets of SNPs for parentage assignment described in sheep. All SNPs from these panels fulfill the QC criteria. Tortereau et al. (2017) reported that the panel of 249 SNPs used for parentage assignment in the French breeds had similar medium MAF values in four Spanish breeds (Churra, Ojalada, Castellana and Rasa Aragonesa) belonging to the Sheep HapMap breeds (Kijas et al., 2012a), suggesting that this set of SNPs should perform well in these breeds. Furthermore, the North American panel of 163 SNPs for parentage testing (Heaton et al., 2014) also found that the Rasa Aragonesa sheep breed had the highest MAF value (0.40) of all breeds.

In the nine North-Eastern Spanish meat sheep populations included in this study, no mendelian inheritance errors were detected in verified family trios or duos for

---

**Table 2.** Major statistics for two parentage panels (French, North American and globally diverse breeds), and a subset of 158 SNPs from the French panel on the 9 Spanish populations: MAF, PI (Probability of identity), PE1 and PE2 (exclusion probabilities considering the exclusion of one or the two parents respectively).

| Breeds                     | MAF     | French panel (249 SNPs) | North American and globally diverse breeds (163 SNPs) | French panel subset (158 SNPs) |
|----------------------------|---------|-------------------------|------------------------------------------------------|-------------------------------|
|                            |         | 1-PE1 | 1-PE2 | 1-PE1 | 1-PE2 | 1-PE1 | 1-PE2 | 1-PE1 | 1-PE2 | 1-PE1 | 1-PE2 |
| Anotana                    | 0.42    | 2.36E-102 | 4.28E-14 | 1.53E-35 | 0.40 | 7.88E-65 | 4.96E-09 | 6.70E-23 | 0.43 | 1.09E-64 | 2.38E-09 | 5.90E-23 |
| Churra Tensina             | 0.39    | 5.02E-102 | 3.29E-14 | 1.17E-35 | 0.37 | 2.11E-63 | 1.95E-08 | 2.75E-22 | 0.41 | 3.38E-65 | 4.62E-09 | 1.03E-22 |
| Xisqueta                   | 0.43    | 1.07E-100 | 4.62E-14 | 1.63E-35 | 0.40 | 1.14E-64 | 6.13E-09 | 7.83E-23 | 0.44 | 2.57E-66 | 2.20E-09 | 5.58E-23 |
| Navarra                    | 0.43    | 1.23E-98 | 1.34E-13 | 4.18E-35 | 0.40 | 8.88E-65 | 5.68E-09 | 7.02E-23 | 0.44 | 2.01E-65 | 1.98E-09 | 5.08E-23 |
| Rasa Aragonesa             | 0.42    | 2.75E-100 | 1.38E-13 | 4.61E-35 | 0.40 | 9.57E-66 | 4.79E-09 | 6.08E-23 | 0.43 | 1.22E-64 | 2.44E-09 | 6.06E-23 |
| Raya Bihiliana             | 0.40    | 1.44E-100 | 9.67E-14 | 3.27E-35 | 0.38 | 4.60E-62 | 1.35E-08 | 1.67E-22 | 0.42 | 4.94E-64 | 3.84E-09 | 8.85E-23 |
| Maelana                    | 0.40    | 2.75E-100 | 1.38E-13 | 4.61E-35 | 0.38 | 7.39E-63 | 1.65E-08 | 2.26E-22 | 0.42 | 1.58E-64 | 3.24E-09 | 7.66E-23 |
| Ojinegra                   | 0.41    | 1.44E-100 | 9.67E-14 | 3.27E-35 | 0.39 | 7.82E-64 | 1.01E-08 | 1.33E-22 | 0.42 | 3.50E-65 | 2.79E-09 | 6.76E-23 |
| Cartera                    | 0.41    | 2.61E-102 | 9.58E-14 | 3.26E-35 | 0.39 | 2.98E-64 | 1.01E-08 | 1.38E-22 | 0.43 | 2.38E-66 | 2.67E-09 | 6.56E-23 |
both panels. SNPs were in Hardy-Weinberg equilibrium. There were not any uninformative SNPs in each breed group (MAF=0) in any breed group. The average MAF for the sets of 249 and 163 SNPs were 0.41 ± 0.01, and 0.39 ± 0.01, respectively. The set of 249 SNPs had better diversity, PI, PE1 and PE2 values than the set of 163 SNPs (Table 2). PI, PE1 and PE2 values are highly dependent on the number of SNPs (Jamieison & Taylor, 1997), but Tortereau et al. (2017) demonstrated that these values were better on these populations with 150 SNPs randomly selected from the French panel than those of the North American panel.

For the reasons described above, we decided to select a reduced panel of 158 SNPs from the French panel for a cost-effective genotyping. Only, SNPs with a MAF value greater than 0.3 and with a call rate > 0.97 in all the nine populations were retained. The SNPs were distributed over the 26 autosomes. The major statistics for this panel of 158 SNPs (MAF, PI, PE1 and PE2) are shown in Table 2. The names, MAFs, and other features of the SNPs of each panel are shown in Tables S1-S3 [suppl]. The average MAF for the set of 158 SNPs was 0.42 ± 0.01, having better values than the other two sets of SNPs. Slightly better values were found for the reduced panel (158 SNPs) compared to the American one (163 SNPs) for the PI, PE1 and PE2 values, although Anso tana and Rasa Aragonesa showed lower PI values with the set of 163 SNPs. At the population level, the lowest and greatest average MAF values were obtained respectively in Churra tensina, and in Xisqueta and Navarra breeds whatever the panel. In general, all breeds showed good PI, PE1, and PE2 values. For the set of 158 SNPs, the probability (PI) that two randomly selected individuals have identical genotypes within a breed was very low, reaching its lowest and highest values in the Cartera (2.38E-66) and the Roya Bilbilita populations (4.94E-64), respectively. However, in the Rasa Aragonesa breed the lowest PI value was found with the set of 163 SNPs (9.57E-66) compared to the set of 158 SNPs (1.22E-64).

The set of 158 SNPs was also used to perform parentage assignment validation using KASP technology. Furthermore, the 15 functional SNPs were also genotyped in conjunction with those used for parentage assignment for a total of 173 SNPs. KASP technology was chosen because it is a very cost-effective genotyping platform. In this sense, the total cost per sample for a set of 192 SNPs assay (DNA extraction and genotyping a maximum of 192 SNPs) was €9 when dealing with more than 1,500 individuals (all-inclusive service from the LGC, Genomics Hoddesdon, UK). The price goes down around €2 when genotyping more than 3,000 samples. Five SNPs from the reduced panel failed or had a call rate <0.95 in KASP genotyping. However, MAF, PI, PE1 and PE2 had similar values (Table S4 [suppl]). Functional SNPs were genotyped successfully. For example, we could genotype efficiently for the numerous alleles of the PRNP gene (Table 1) at codons 136 (p.A136V,T), 141 (p.L141F), 154 (p.R154H) and 171 (p.Q171R,H,K), identifying 3, 2, 2, and 4 alleles for each codon, respectively. This validation was performed in 12 commercial farms from three different breeds. Farmers declared a proportion of putative sires sampled from the farm because not all the putative males were available, mainly because some sires were dead. Table 3 shows the assignment rate in different farms from the three breeds. As expected when the list of putative sires was completely (or almost) genotyped in a farm, a very high assignment rate was obtained. In two farms, a 100% assignment rate was achieved. In general, the assignment rate is highly dependent to the percentage of putative sires genotyped. We only found one out of 2,018 replacements ewes (farm G) with two possible parents, a father-offspring pair. This problem has been previously pointed by Tortereau et al. (2017) recommending to genotype at least 180 SNPs given the number of false-positive results when the dam is not genotyped and the true sire is not among the candidate sires or are highly-related. Because this is an open genotyping platform we could complete the panel with more SNPs to increase the parentage assignment power; or add new validated functional SNPs.

The total cost per sample for this set of 173 SNPs for parentage assignment and genotyping some functional genes (the same price is for the genotyping a maximum of 192 SNPs) is similar to those used with microsatellites. In this way, this panel is routinely used in Rasa Aragonesa and Ojinegra sheep breeds for parentage assignment and genotyping of functional SNPs by KASP. Marker- or gene-assisted selection (MAS/GAS) is been applied in these breeds for pre-selection of replacement animals for increasing frequency of favorable alleles of a major gene, for example PrnP alleles for scrapie resistance or BMP15 alleles for litter size. However, a balance over time between selection for polygenes and the major gene for a given trait is needed to avoid inbreeding, and maintain the genetic variability within the breed.

In conclusion, the described method is successfully used in some meat Spanish sheep breeds, combining the genotyping of SNPs for the parentage assignment with some functional SNPs that can be used for pre-selection of replacement animals. The described method is a cost effective genotyping system, which is routinely used in Rasa Aragonesa and Ojinegra meat sheep breeds in their selection schemes by KASP genotyping technology. In addition, the SNPs for the parentage assignment could be genotyped using other genotyping platforms such as, for example, a custom low density array (these SNPs are included in the Illumina OvineSNP50 BeadChip array) or by Sequenom technology as described by Tortereau et al. (2017).
Acknowledgments

The authors thank to UPRA-Grupo Pastores, ARA-MA, AGROBI, ARACOXI, ATURA, ARANA, ACOAN, AGROJI and ANGORCA breeders associations for the experimental samples.

References

Acín C, Martín-Burriel I, Goldmann W, Lyahyai J, Monzón M, Bolea R, Smith A, Rodellar C, Badiola JJ, Zaragoza P, 2004. Prion protein gene polymorphisms in healthy and scrapie-affected Spanish sheep. J Gen Virol 85: 2103-2110. https://doi.org/10.1099/vir.0.80047-0

Arruga MV, Monteagudo LV, Tejedor MT, Barrao R, Ponz R, 2001. Analysis of microsatellites and paternity testing in Rasa Aragonesa sheep. Res Vet Sci 70: 271-273. https://doi.org/10.1053/rvsc.2001.0473

Barnett, NL, Purvis, IW, van Hest, B, Franklin I, 1999. The accuracy of current dam pedigree recording strategies employed by study Merino breeders. Rising to the challenge: Breeding for the 21st century customer. Assoc Adv Anim Breed Genet, pp: 13:373-376.

Bell AM, Henshall JM, Gill S, Gore K, Kijas JW, 2013. Success rates of commercial SNP based parentage assignment in sheep. Assoc Adv Anim Breed Genet, pp: 20: 278-281.

Bodin L, Di Pasquale E, Fabre S, Bontoux M, Monget P, Persani L, Mulsant P, 2007. A novel mutation in the bone morphogenetic protein 15 gene causing defective protein secretion is associated with both increased ovulation rate and sterility in Lacaune sheep. Endocrinology 148: 393-400. https://doi.org/10.1210/en.2006-0764

Calvo JH, Serrano M, Martinez-Royo A, Lahoz B, Sarto P, Ibañez-Deler A, Folch J, Alabart JL, 2018. SNP rs403212791 in exon 2 of the MTNR1A gene is associated with reproductive seasonality in the Rasa aragonesa sheep breed. Theriogenology 113: 63-72. https://doi.org/10.1016/j.theriogenology.2018.02.013

Calvo JH, Chantepie L, Serrano M, Sarto MP, Iguaucel LP, Jiménez MÁ, Alabart JL, Folch J, Fabre S, Lahoz B, 2020. A new allele in the BMP15 gene (FecXRA) that affects prolificacy co-segregates with FecXR and FecXGR in Rasa aragonesa sheep. Theriogenology 144: 107-111. https://doi.org/10.1016/j.theriogenology.2020.01.010

Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ, 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience 4: 7. https://doi.org/10.1186/s13742-015-0047-8

Clarke SM, Henry HM, Dodds KG, Jowett TWD, Manley TR, Anderson RM, McEwan JC, 2014. A high throughput single nucleotide polymorphism multiplex assay for parentage assignment in New Zealand sheep.

Table 3. Assignment rate of replacement ewes in different farms from the Rasa Aragonesa, Navarra and Cartera breeds. The number of replacement ewes, sires and declared proportion of sires sampled and genotyped are also indicated.

| Breed         | Farm | Replacement ewes | Sires | Assignment rate (%) | Proportion of candidate parents sampled (%) | [1] |
|---------------|------|------------------|-------|---------------------|---------------------------------------------|-----|
| Rasa Aragonesa| A    | 35               | 5     | 100                 | 100                                         |     |
|               | B    | 140              | 26    | 74                  | 80                                          |     |
|               | C    | 160              | 73    | 100                 | 95                                          |     |
|               | D    | 343              | 70    | 74                  | 80                                          |     |
|               | E    | 295              | 56    | 80                  | 80                                          |     |
|               | F    | 125              | 56    | 88                  | 90                                          |     |
|               | G    | 625              | 73    | 86                  | 90                                          |     |
|               | H    | 60               | 25    | 92                  | 90                                          |     |
| Navarra       | I    | 116              | 12    | 67                  | 75                                          |     |
|               | J    | 50               | 10    | 96                  | 95                                          |     |
| Cartera       | K    | 39               | 5     | 87                  | 80                                          |     |
|               | L    | 30               | 17    | 85                  | 80                                          |     |

[1] According to the information provided by farmers.
SNP parentage testing in some North-Eastern Spanish meat sheep breeds

Heaton MP, Harhay GP, Bennett GL, Stone RT, Grosse WM, Casas E, Keele JW, Smith TPL, Chitko-Mckown CG, Laegreid WW, 2002. Selection and use of SNP markers for animal identification and paternity analysis in U.S. beef cattle. Mamm Genet 39: 294-297. https://doi.org/10.1111/j.1365-294X.2007.03089.x

Kijas JW, Lenstra JA, Hayes B, Boitard S, Neto LR, Cristobal MS, Servin B, McCulloch R, Whan V, Gietzen K, et al., 2012a. Genome-wide analysis of the world’s sheep breeds reveals high levels of historic mixture and strong recent selection. PLoS Biol 10: e1001258. https://doi.org/10.1371/journal.pbio.1001258

Kijas JW, Lenstra JA, Hayes B, Boitard S, Porto Neto LR, San Cristobal M, Servin B, McCulloch R, Whan V, Gietzen K, et al., 2012b. Genome-wide analysis of the world’s sheep breeds reveals high levels of historic mixture and strong recent selection. PLoS Biol 10: e1001258. https://doi.org/10.1371/journal.pbio.1001258

Martinez-Royo A, Jurado JJ, Smulders JP, Marti JI, Alabart JL, Roche A, Fantova E, Bodin L, Mulsant P, Serrano M, Folch J, Calvo JH, 2008. A deletion in the bone morphogenetic protein 15 gene causes sterility and increased prolificacy in Rasa Aragonesa sheep. Anim Genet 39: 294-297. https://doi.org/10.1111/j.1365-2052.2008.01707.x

Meuwissen T, Hayes B, Goddard M, 2013. Accelerating improvement of livestock with genomic selection. Annu Rev Anim Biosci 1: 221-237. https://doi.org/10.1146/annurev-animal-031412-103705

Moum T, Olsaker I, Hopp P, Moldal T, Valheim M, Moum T, Benestad SL, 2005. Polymorphisms at codons 141 and 154 in the ovine prion protein gene are associated with scrapie Nor98 cases. J Gen Virol 86: 231-235. https://doi.org/10.1099/vir.0.80437-0

Raoul J, Palhiere I, Astruc JM, Elsen J M, 2016. Genetic and economic effects of the increase in female paternal filiations by parentage assignment in sheep and goat breeding programs. J Anim Sci 94 (9): 3663-3683. https://doi.org/10.2527/jas.2015-0165

Safariand A, Javanmard A, Safdari M, 2011. Parentage verification and identity test of Ghezel sheep using microsatellite markers. Afr J Biotechnol 10: 5815-5819. https://doi.org/10.5897/AJB10.1914
Salces-Ortiz J, Ramón M, González C, Pérez-Guzmán M D, Garde J, García-Álvarez O, Maroto-Morales A, Calvo JH, Serrano M, 2015. Differences in the ovine HSP90AA1 gene expression rates caused by two linked polymorphisms at its promoter affect rams sperm DNA fragmentation under environmental heat stress conditions. PLoS One 10. https://doi.org/10.1371/journal.pone.0116360

Schütz E, Brenig B, 2015. Analytical and statistical consideration on the use of the ISAG-ICAR-SNP bovine panel for parentage control, using the Illumina BeadChip technology: Example on the German Holstein population. Genet Sel Evol 47. https://doi.org/10.1186/s12711-014-0085-1

Sider LH, Heaton MP, Chitko-Mckown CG, Harhay GP, Smith TP, Leymaster KA, Laegreid WW, Clawson ML, 2013. Small ruminant lentivirus genetic subgroups associate with sheep TMEM154 genotypes. Vet Res 44: 64. https://doi.org/10.1186/1297-9716-44-64

da Silva EC, McManus CM, de Paiva Guimarães MPSLM, Gouveia AMG, Facó O, Pimentel DM, Caetano AR, Paiva SR, 2014. Validation of a microsatellite panel for parentage testing of locally adapted and commercial goats in Brazil. Genet Mol Biol 37: 54-60. https://doi.org/10.1590/S1415-47572014000100010

Souza CA, Paiva SR, McManus CM, Azevedo HC, Mariante AS, Grattapaglia D, 2012. Genetic diversity and assessment of 23 microsatellite markers for parentage testing of Santa Inês hair sheep in Brazil. Genet Mol Res 11: 1217-1229. https://doi.org/10.4238/2012.May.8.4

Tortereau F, Moreno CR, Tosser-Klopp G, Servin B, Raoul J, 2017. Development of a SNP panel dedicated to parentage assignment in French sheep populations. BMC Genet 18: 50. https://doi.org/10.1186/s12863-017-0518-2

Visscher PM, Woolliams JA, Smith D, Williams JL, 2002. Estimation of pedigree errors in the UK dairy population using microsatellite markers and the impact on selection. J Dairy Sci 85: 2368-2375. https://doi.org/10.3168/jds.S0022-0302(02)74317-8

Visser C, Van Marle-Köster E, Friedrich H, 2011. Parentage verification of South African Angora goats, using microsatellite markers. S Afr J Anim Sci 41. https://doi.org/10.4314/sajas.v41i3.7

Werner FAO, Durstewitz G, Habermann FA, Thaller G, Krämer W, Kollers S, Buitkamp J, Georges M, Brem G, Mosner J, Fries R, 2004. Detection and characterization of SNPs useful for identity control and parentage testing in major European dairy breeds. Anim Genet 35: 44-49. https://doi.org/10.1046/j.1365-2052.2003.01071.x