Small Ruminant Research 149 (2017) 85–90

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Research Paper

Lamb meat traceability: The case of Sambucana sheep

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ARTICLE INFO

Article history:
Received 30 May 2016
Received in revised form 31 October 2016
Accepted 26 January 2017
Available online 30 January 2017

Keywords:
Genetic traceability
Meat
Sheep
Sambucana breed

ABSTRACT

Genetic traceability has a key role in the product certification, but it is rarely implemented in sheep so far, especially in the fresh meat sector. In this study, the case of the Sambucana sheep is analysed with the aim of developing a genetic system able to certify the origin of its traditional product, the Sambucano lamb, protected by a registered trademark. A set of 14 microsatellite markers was identified as an efficient tool to genetically discriminate the Sambucana sheep from other breeds potentially involved in mislabelling and to allow for an effective allocation test of meat cuts labelled as 'Guaranteed Sambucano lamb'. The paternity test proved to be an additional means to improve the reliability of the control. The traceability system here described is easy to implement in local minor sheep breeds and is recommended in the framework of meat certification.

1. Introduction

The conservation of valuable local breeds is a worldwide recognized imperative to contrast the loss of genetic resources in livestock species, which are of vital importance to agriculture, food production, rural development and the environment (FAO, 2007). Of the several actions suggested to preserve the existing biodiversity (FAO, 2013), the valorization and protection of typical products derived from local minor breeds is now playing a prominent role, for increasing consumer awareness of food nutritional properties and safety. Also socio-cultural and ethical implications lead the consumers to choose products derived from traditional breeds, because they are linked to a specific area of origin, and so representative of historical and geographical identity (Montossi et al., 2013). The increased interest in the origin of lamb meat for the consumer decisions has been demonstrated by several studies, especially in Europe (Font i Furnols et al., 2011; Hersleth et al., 2012). Therefore, the link 'breed-product' can become an effective means to satisfy the consumer's expectations, which in turn can contribute to improve the self-sustainability of the breed.

Italy has a wide variety of local breeds, from which an extraordinary richness of typical products are derived. In order to protect their peculiarity, many of them have obtained the EC labels (Protected Designation of Origin, PDO, or Protected Geographical Indication, PGI), and many others are recognized by registered trademarks. Most of them concern dairy products or meat-derived products, while very few concern fresh meat.

One interesting example in this sector is represented by the Sambucana sheep, an autochthonous breed traditionally reared in Cuneo province (Piedmont Region, Italy), mainly for meat production, with the commercialization of lambs weighing between 18 to 20 kg. The massive crossing with the Biellese breed in the middle of the last century caused a drastic decline of the Sambucana, with 1400–1600 heads and about 60 purebred rams estimated at the end of the '70 s (Brooke and Ryder, 1978). To avoid its definitive loss, a conservation programme was started in 1985, which included the foundation of the Breeders' Association in 1988 and the establishment of the Centre for the ram selection, as well as many other supporting activities. As a result the breed size increased, with 126 rams and 2733 ewes registered in 2014, distributed in 49 herds in the Piedmont Region (Asso.Na.Pa., 2014). The conservation of the breed took great advantage from the valorization of the meat production, resulting in the inclusion of the Sambucano lamb in the Traditional Farming Products of the Piedmont Region (Regione Piemonte, 2016) and in the Slow Food Presidia (Slow Food Foundation, 2016). In 1992, the 'Guaranteed Sambucano lamb'

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http://dx.doi.org/10.1016/j.smallrumres.2017.01.013
0921-4488/

trademark was registered at the Chamber of Commerce of Cuneo, and managed by a farmers' cooperative framework dealing with marketing the product.

In this context, the certification of the breed of origin becomes necessary for the sake of product protection, because the high commercial value of the labelled product could bring about the risk of fraudulent mislabeling. In the case of fresh meat, unintentional errors can also occur along the production chain, from the birth of the animal to the butcher's shop, passing through several steps where the animal identification may be replaced by another identification.

Among the different tools for breed traceability, the DNA-based methods are widely recognized as the most powerful (Dalvit et al., 2007; Scarano and Rao, 2014; Sentandreu and Sentandreu, 2014), because they can be applied at any stage of the production chain to assign a given product to a given breed on the basis of their genetic similarity using a set of markers. So far, breed allocation based on molecular markers has been profitably applied for meat traceability in different animal species, including cattle (Dalvit et al., 2008; Rogberg-Muñoz et al., 2016) and pigs (García et al., 2006; Oh et al., 2014), while in sheep molecular traceability systems are still difficult to implement for the high cost of genotyping relative to the economic value of the single animal, especially in minor breeds, which usually suffer from lack of funds.

The Sambucana breed is an interesting case-study also because the traceability system could benefit from the distinctive organization of the supply chain, as the production cycle occurs in a well-defined geographic area, data are recorded along the whole chain and the slaughtered lambs derive from a limited number of rams. In this situation, the establishment of a database with the genetic profiles of the used rams could be useful to implement a paternity test in order to verify if the profile of a meat cut is compatible with one of the Sambucana rams, hence confirming the assignment to the breed.

Based on all these considerations, the aim of our study was to develop a genetic traceability system for fresh sheep meat applied to the control of the 'Guaranteed Sambucano lamb' supply chain, by investigating the different aspects concerned: i) resolving power of a set of microsatellite markers; ii) genetic differentiation of the Sambucana breed from other Italian sheep breeds, including those potentially involved in the traceability system; iii) breed assignment and paternity test of meat cuts purchased in different shops and labelled as 'Guaranteed Sambucano lamb'.

2. Material and methods

2.1. Samples and DNA extraction

Blood samples were collected from Sambucana subjects (n = 58) and from other nine Italian sheep breeds purposely chosen: Biellese (n = 57), reared in the same Region and often used for crossing, potentially mislabeled as Sambucana; Sarda (n = 20), also potentially involved in mislabeling, being the most widespread Italian breed and marketed in the whole country; Frabosana (n = 22), Saltasassi (n = 13) and Savoiarda (n = 20), minor Piedmontese breeds, for which possibility of crosses with breeds reared in the same area exists; Bergamasca (n = 23), often used in the past for crossbreeding to improve the Sambucana for meat production; Appenninica (n = 25) and Merinizzata (n = 22), meat breeds reared in a different area; Comisana (n = 30), as representative of a dairy breed. Individuals as little related as possible were sampled. In addition, blood samples were collected from 186 Sambucana rams, representing most of the males used for reproduction in the production area, and meat cuts labelled as 'Guaranteed Sambucano lamb' (n = 49) were anonymously purchased from butcher shops located in the Piedmont region.

Genomic DNA was extracted from blood and meat samples using respectively the NucleoSpin Blood and the NucleoSpin Tissue kits (Macheray-Nagel, Düren, Germany), according to the manufacturer's instructions.

2.2. Markers and genotyping

Fifteen microsatellite markers, included in the panels suggested by the International Society for Animal Genetics (ISAG) and/or FAO, were amplified: OarCP049, OarFCB304, CSRD247, INRA063, HSC, MAF214, McM527, OarFCB020, D5S2, MAF065, INRA023, TGLA53, ETH10, ETH225, BM1824 (FAO/ISAG, 2004). Fragments were resolved in a DNA sequencer ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). The allele size was assigned using the Genemapper 4.0 software (Applied Biosystems, Foster City, CA) and the allele nomenclature was standardized using reference samples from the Comparison Tests organized by the ISAG.

2.3. Statistical analysis

The marker informativeness was evaluated across breeds by computing the number of observed and effective alleles using the Popgene software version 1.32 (Yeh et al., 1999). The FSTAT 2.9.3.2 software (Goudet, 2002) was used to analyse the single-locus F_{ST} and the linkage disequilibrium between loci. The Polymorphism Information Content (PIC) of each marker was also calculated (Nagy et al., 2012). The within-population variability was estimated by the allele frequencies, observed number of alleles per locus, allelic richness, as well as observed and expected heterozygosity, using the FSTAT 2.9.3.2 software (Goudet, 2002).

Using the same software the between-breed diversity was evaluated by the pairwise fixation index (F_{ST}) and the global F_{ST}. The sequential Bonferroni correction (Rice, 1989) was applied to correct for the effects of multiple tests. To analyse in more details the population differentiation the Structure 2.3.4 software (Pritchard et al., 2000) was also employed, conducting the analysis with the admixture model, Locprior option (considering the sampling location, basically the Region where the breed is reared), correlated allele frequencies, burn-in 200,000 and MCM iteration of 500,000. The results were graphically displayed using the DISTRUCT program (Rosenberg, 2004). The same procedure was used for the assignment of declared 'Guaranteed Sambucana lamb' meat samples, but in this case only the Piedmontese breeds as well as Bergamasca and Sarda were included in the test. In addition, the Cervus 3.0.3 software (Kalinowski et al., 2007) was employed to calculate for each meat sample the likelihood of paternity assignment to one of the tested rams.

3. Results and discussion

3.1. Marker statistics

The number of observed and effective alleles, together with the Polymorphism Information Content and the Fixation index of the 15 markers used are reported in Table 1. A total of 198 alleles were observed, with a mean of 13.2/locus, ranging from 4 (ETH10) to 21 (CSRD247 and INRA063). The number of the effective alleles was in general much lower (1.19 ÷ 7.83, mean value 4.60), for the very low frequency of many alleles. Fourteen loci had PIC values exceeding 0.5 (0.59 ÷ 0.86), which was indicated as the limit for considering a marker highly informative (Botstein et al., 1980), while ETH10 had a value of 0.17, so poorly contributing to the characterization of the within-breed variation, as shown for other sheep breeds also (Lasagna et al., 2011). Moreover, the F_{ST} value for ETH10