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Individual traceability of Iberian pigs: Electronic identification and validation using molecular markers

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SUMMARY – Full traceability in the animal food supply chain is increasingly demanded by consumers as an essential tool for monitoring food safety and quality. Traditional tagging systems present several disadvantages in Iberian pigs because of their production system. A critical aspect of electronic identification is the injection site, because transponders will migrate in the fat tissues in heavy pigs. More than 1700 Iberian piglets were injected intraperitoneally with numbered transponders. Two biological samples (blood from the piglets and fresh meat samples from the carcasses) were used to test individual traceability. Preliminary results showed that only 3.58% of the transponders were not detected at the slaughterhouse. 99% of the detected devices were found at the abdominal cavity and the remaining 1% was found on the carcasses. The validation of the two biological samples, made using a panel of 8 microsatellites, showed a total agreement between the two kinds of samples.

Keywords: Traceability, Iberian, electronic-identification, microsatellites.

RESUME – *"Traçabilité individuelle chez le porc ibérique : identification électronique et validation à l'aide de marqueurs moléculaires". La traçabilité complète dans la chaîne alimentaire est une demande qui augmente de la part des consommateurs, pour garantir la qualité et la sécurité des aliments. Chez le porc Ibérique, les systèmes traditionnels d'identification présentent beaucoup de problèmes liés au système de production. Dans cette race l'identification électronique présente un aspect critique : le lieu d'injection des micropuces, parce que chez les porcs lourds elles peuvent migrer à travers les tissus gras. Plus de 1700 porcelets ibériques ont été injectés par voie intrapéritonéale avec des micropuces, et deux échantillons biologiques (de sang chez les porcelets et de muscle dans les carcasses) ont été pris pour vérifier la traçabilité individuelle. Les résultats préliminaires montrent que seulement 3,58% des micropuces ne sont pas détectées à l'abattoir. Quatre-vingt dix neuf pour cent des micro chips détectées se trouvaient dans la cavité abdominale tandis que 1% étaient dans la carcasse. La validation individuelle des deux échantillons biologiques grâce à un ensemble de 8 microsatellites a montré un accord total entre les deux types d'échantillons.*

Mots-clés : *Traçabilité, Ibérique, identification électronique, microsatellites.*

Introduction

Full traceability in the animal food supply chain is increasingly demanded by consumers as an essential tool for monitoring food safety and quality. In order to satisfy this demand is necessary to have an identification system that allows tracing raw animal material from their origin to the marketed products. Traditional tagging systems present several difficulties mainly in Iberian pigs because of their extensive production system (López-Bote, 1998). In this case, systems based on electronic identification (EID) could be a good alternative. Electronic identification of Iberian pigs started more than 10 years ago using diverse procedures. However, a critical aspect of this kind of identification is the injection site. Previous results showed that ear-base implementation presents some problems (Roca *et al.*, 1998) mainly in heavy pigs, because they are extremely fats and the transponders will migrate along the fat tissues, being more difficult their recovery from the carcasses (Caja *et al*., 2003).

DNA profile could be used as a consistent method to audit the tracing-back of the identity of animals, carcasses and meat cuts in the whole meat industry process, at reasonable cost and response time.

A double system based on electronic identification and DNA profiling for tracing animals and meat, has been developed in a EU FAIR 5th project (QLK1-CT2001-02229).

The objectives of this work were: (i) to test the abdominal cavity as the optimal injection body site for electronic identification in Iberian pigs; (ii) to detect the weak points in the production cycle from farm to the abattoir and the market; (iii) to check the readability, losses and breakages in the abattoir; and (iv) the application of a panel of molecular markers to the verification of electronic identification.

Material and methods

Animals

Electronic identification was carried out in a total number of 1700 Iberian male castrated piglets from five different farms. Iberian piglets were injected in the intraperitoneal cavity with *Tiris* HX 32 x 3.8 mm transponders, using a multi-shot injector (*Tiris*) equipped with a multiple use 50 x 4.8 mm needle. Before each injection the body site was disinfected with an iodine solution and the needle was cleaned with the same iodine solution described above after each application. The injection was made on the left side of the animal, at 1 cm from the ventral line and 2-8 cm caudally to the navel, in a perpendicular direction towards the abdominal cavity (Hernandez-Jover *et al*., 2003). The transponder must be injected between the ventral line and the mammary blood vessel to avoid possible bleedings. The piglet identification was immediately read and recorded after the injection with a hand-held transceiver to detect their readability. The injection of the transponders took place five/ten days after the weaning. In Iberian pigs the weaning usually took place at around 36 days old.

At the moment of the transponder injection samples of blood (impregnated in FTA cards, Whatman International Ltd., UK) of all piglets were taken. Ear biopsies from a small sub sample of pigs (n=20) were also taken. FTA card were stored at room temperature meanwhile the biopsies were stored at -20ºC. DNA from FTA cards was extracted following the manufacturer recommendations and DNA extraction from meat samples was done using the Cell and Tissue – Puregene™ DNA Purification Kits, Gentra Systems, Minneapolis.

The pigs were fed, under restricted feeding regime and in semi-extensive conditions, until they have around 14 moths old. When they reach this age they were moved to another farms, fattened *ad libitum* and under extensive conditions, until their live weigh was around 160-180 kg and they were at least 18 moths old. All the animals were slaughtered in a commercial slaughterhouse. Slaughter included several processes: CO or electrical stunning, bleeding, scalding, peeling, flaming and evisceration. Carcasses were transported to meat factories for their processing in order to obtain the commercial cuts or transformed products.

The verification of the correctness of the meat identification was made in a random and representative sample (5%) of the electronically identified animals. For this purpose a small piece of meat has been taken in the *Longissimus dorsii* muscle at the meat industry.

Molecular markers

Different subsets of microsatellites, recommended by the International Society of Animal Genetics (ISAG), with wide international use in several pig breeds, have been tested for informativity. In addition to informativity other criteria as accuracy of allele identification, possibility of multiplexing, lack of null alleles and chromosomal position has been considered for their selection.

A panel of eight pig microsatellite markers, that provides a power of exclusion with a probability greater than 99.9 has been shown enough for initial analysis of individual identification (Table 1). The

Table 1. Panel of microsatellites

Microsatellite	Chromosome	Size (bp)	Alleles
SW240	2	93-112	8
S0002	3	198-212	5
S0005	5	209-250	12
SW632	7	169-177	9
S0225	8	168-190	7
SW911	9	153-167	7
SW857	14	145-158	6
SW936	15	90-116	10
SW24	17	93-118	7

accuracy of the panel has been evaluated in a ring-test involving different laboratories and pig breeds. Whit this panel of markers the genotyping will be carried out using an automated DNA sequencer in only one step combining the size of the alleles and the fluorescence used. A complementary panel of four microsatellites was established for additional analysis if necessary (Table 2).

Table 2. Complementary panel of microsatellites

Microsatellite	Chromosome	Size (bp)	Alleles
S0155	1	146-160	5
SW72	3	106-116	7
SW122	6	106-116	5
S0026	16	92-103	5

Results and discussion

The control of the complete traceability has been preformed in two groups of animals, composed by 100 and 200 pigs. The injected transponders were tested on field conditions, two weeks before the pigs were translated to the abattoir. At this moment a 3.58% of the transponders were not detected. All the transponders read on field conditions were also detected at slaughterhouse, after bleeding, scalding, peeling and flaming. After evisceration all the carcasses were checked again and the 99% of the devices were detected and located in the abdominal cavity (at the aumentum major) or in the floor. Only a 1% of the transponders were detected into the carcasses. Before the device recovery the identification of transference from the animal to the carcass was carried out manually.

Eighteen double samples (FTA card blood – meat), that represent a 6% of the pigs injected in these first two batches, have been tested using the panel of eight microsatellites selected for pigs. The validation of all the samples was excellent because it showed a complete correspondence between the two kinds of biological samples. Only the first panel of markers was necessary to check the traceability. In the Fig. 1 the electropherogame corresponding to three out of the eight microsatellites (SW936, SW857 and S0005) for two of the analysed pigs (identification codes 20022 and 20061) is showed as an example.

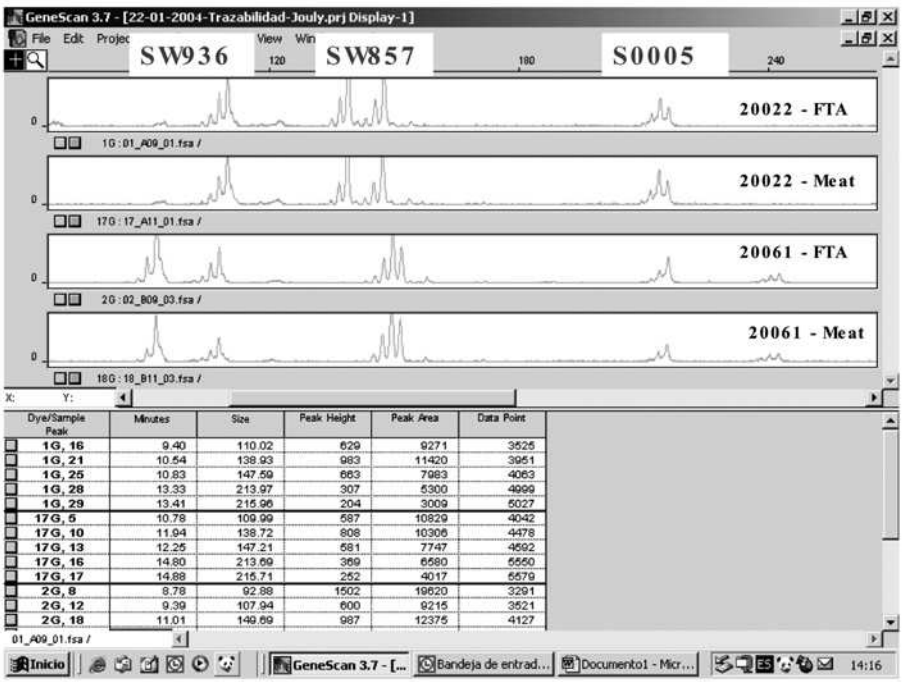

Fig. 1. Electrophoregame of the microsatellites SW936, SW857 and S0005 and the samples of the 20022 and 20061 pigs.

Although the present day results are preliminary, since they correspond only to the first groups of Iberian pigs under control, they seem very promising. In spite of the long production cycle of Iberian pigs, which are slaughtered with at least 18 months of age, only a percentage of 3.58% of transponders injected in these trials were no detected. This study will be completed along the next months, and more fimly founded conclusions will be then available.

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