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Electronic intraperitoneal identification and DNA analysis *in vivo* and post slaughter in swine

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SUMMARY – 71 piglets (1d to 4d aged and at 2.9 to 3.7 kg weighed) after being identified by eartags were divided into two groups: 52 piglets (T group) were also electronically identified by injectable transponders HDX 32.5×3.8 mm 134.2 kHz and sampled for DNA analysis from auricle tissue; 19 piglets (C group) as control. Averaged time for transponder's application and auricle sampling took 2'±9"/pig. On 4 T group animals, x-ray analysis was carried out at 0d, 28d and 70d after EID to detect its position in abdomen. Live weights: 122.1±4.0 kg *vs* 123.6±3.6 kg; mean daily gain: 624±0.9 gr *vs* 632±1.7 gr; carcass yield: 78.2% *vs* 77.6% of T and C group pigs, respectively slaughtered at 203d, showed no statistical significance. Eartag loss on pigs from both groups was 11.2% at 28d, 14.1% 70d and 22.5% 203d. *In vivo* and *post mortem* transponders readability was 100%. Transponders recovery in the abattoir was 100%: 75% found free in abdomen, 17.3% embedded in omentum fat and 7.7% extraperitoneal. Genetic profiles by 6 microsatellites among FAO-ISAG panel confirmed the correspondence between in vivo and post mortem samples. DNA analysis on muscles randomly sampled from 11 T group carcasses matched with genetic profiles from auricles, while muscles' genetic profiles from 7 C group carcasses showed genetic non identity.

INTRODUCTION – In 2000, Merks (**5**) pointed out three milestones laid down in pig production (Crossbreeding; Family selection; DNA analysis) so far; at present, we believe that a fourth milestone can match with traceability. The food supply chain traceability involves all the stakeholders at each step, starting from farmers, whose major needs concern the meeting of EU Regulations (178/2002) and the origin protection of their products from attempts of fraud on the market, up to consumers who need to be assured about the product they are buying. RFID technology for individual animal identification (**2**) could be a useful tool to achieve this twin goal. On the basis of prior experimental trials (**8**) in the niche meat chain of "Suino Tipico Sardo", lead on piglets slaughtered when 28d aged, the present paper aims to broaden the experimental trial in pigs of 203d of age. Moreover, the DNA analysis concerned the sampling of muscle tissue from the meat product aiming to traceback from the meat product to the live animal.

MATERIALS AND METHOD – 71 suckling piglets at 1-7 days old and at an average weight ranging between 2.9 and 3.7 kg have been segregated into 2 experimental groups: 52 were electronically identified (EID) by means of intraperitoneal injection of transponders, according to Caja et al. technique (**3**) and belonged to the T group. All the HDX encapsulated in bioglass transponders 32.5×3.8 mm, 134.2 kHz (TIRIS™) used agreed with ISO11784 and 11785 Standards for animal identification. Together with the

electronic identification, each piglet was also identified by means of ear tag. Transponder's and ear tag's codes have been paired directly on a handy reader Gesreader 2S ISO® (Gesimpex Com. S.L., Barcelona, Spain). 19 piglets were identified by eartags only and belonged to the C group, kept as control. Times of electronic identification performing/piglet in farm were noted down. At ear tagging of T group animals, a first auricle sample (*in vivo*) was stored in a tube 2 ml and then frozen at -20 °C in the laboratory, for DNA extraction. *In vivo,* the anagraphical identity (eartag loss and working transponders rate) and the post-surgery follow up of animals (bleeding, hernia occurrence and recovery of the wound) after the intraperitoneal injection of the transponders were carried out in farm at 0 d, 28d, 70d and 203d after identification. By the way, on 4 random T group animals, x-ray analysis were carried out to check the transponder position in the abdomen cavity and reveal any radiologic warning signal of harm on viscera caused by the transponder presence. Animals welfare was also catalogued by an ethogram development (**1**), by video recording T and C group animal's behaviour and coping with environment during the next 24 h after identification. *Post mortem* in the abattoir*,* the presence of the ear tags and the functioning of the transponders were checked. Across the slaughtering chain, eartags and transponders codes were read after 6 steps: 1. at unloading of animals from the mean of transport to the boxes before slaughter; 2. after the electric stunning (1.1 sec, 100 Volts and 0.9 Å); 3. after bleeding; 4. after singeing to remove hairs by a gas blowpipe; 5. after washing under high pressured hot/cold water (60 °C – 15 °C); 6. after evisceration.

Readability (R%), defined as ability of transponder to function in the animal's body and possibility to be detected by handy reader, was calculated by the formula:

R (%) = (number of read transponders / number of piglets with transponder)*100

After evisceration, the carcasses from both groups underwent veterinary inspection. Then, the fatness was visually evaluated, the carcass has been weighed and the carcass yield calculated. *In vivo* and *post mortem* performance of animals from both the experimental groups were statistically compared (SAS/Stat software). Each transponder recovered at the disembowelling was enclosed manually to the respective carcass that moved on the slaughtering chain to the freezing chamber. Time of transponder recovery from each carcass were noted down. A second auricle sample from each carcass was kept according to the electronic code that identified the carcass and stored as the first sample. In the freezing chamber, a third sample of muscle tissue from 11 EID carcasses and 9 C carcasses was stored stored as the first and second samples, for genetic profile comparing. From *in vivo* and *post mortem* samples of each pig, the DNA was amplified through PCR, deploying 6 microsatellites [SW122; IGF1; SW240; S0068; SW72; S0225], among those suggested for swine species of FAO/ISAG panel (**9**). PCR products have been separated and evidenced by capillary electrophoresis by ABI PRISM 3100 Genetic Analyser (Applied Biosystem) and data elaborated by the software of the equipment. The genetic profiles from the 2 auricle samples of each pig were compared to check potential non identity cases. The genetic profiles obtained from muscle DNA from EID carcasses and anonymous carcasses (C group) were compared to the genetic profiles from *in vivo* auricle sample too.

RESULTS AND DISCUSSION – Averaged time required to performe the intraperitoneal injection and auricle sampling/storing in farm by a skilled technician was 2' 9"/piglet. At clinical checks and welfare monitoring of piglets during the 24 h after identification, no harms or behavioural modifications were revealed at the ethogram development between the groups. The recovery of the wound took a week time, on average and no complications (infections, myasis, post surgery wound dehiscence) occurred. The radiographies carried out at 0d, 28d and 70d allowed to follow the positioning of the transponder in the abdomen cavity. No radiologic warning signals of harm on viscera were seen.

Table 1 shows live weights, the average daily gain (T *vs* C) and the readability of transponders' and ear tags' codes.

Table 1 – *In vivo* items (T *vs* C), readability (T group) and eartags loss (T+C group)

	Live weight (g) (T group)	Live weight (g) (C group)	Significance	Readability (T group)	Eartag loss (T+C group)
Number of animals	52	19	P<0.01	52	71
Transponder application (1 d after)	3130 ± 379	3169 ± 366	n.s.	100 %	0 %
Transponder application (28 d after)	11015 ± 1115	11230 ± 504	n.s.	100 %	11.2 %
Transponder application (70 d after)	30247 ± 2676	33453 ± 2374	n.s.	100 %	14.1 %
Transponder recovery (203 d after at abattoir)	122090 ± 3997	123623± 3612	n.s.	100 %	22.5 %
Averaged daily gain (1-203 d)	624 ± 0.9	632 ± 1.7	n.s.		

Data reported about *in vivo* performance of animals from both the experimental groups agree with Pinna et al. (**7, 8**). It is to point out that while *in vivo* readability of transponders was 100% at each control in farm, an increasing eartag loss total rate reached the 22.5% of animals in a 203d period, meaning that these animals lost their identification at arriving at the abattoir. The reading of the transponder code by handy reader was easy and immediate. While the reading of the code of the eartag constantly took a longer time because of the need to restrain the animal and to remove the dirt accumulated on the eartag itself. Table 2 shows the readability of the transponders across the slaughtering chain, the transponder's collection and the *post mortem* productive performance.

Table 2 – *Post mortem* items (T *vs* C group), readability and recovery of transponders (T group) across the slaughtering chain

	Transponder readability	Transponder recovery	Productive performance (T group)	Productive performance (C group)	Significance
Number of animals	52	52	52	19	P<0.01
Carcass averaged weights			95545±6967 g	94700±3466 g	n.s.
Carcass warm yield			78.2 %	77.6 %	n.s.
1st step: Electric stunning	100 %				
2nd step: Bleeding	100 %				
3rd step: Scalding	100 %	Eartag trace stop			
4th step: Washing	100 %				
5th step: Singeing	100 %				
6th step: Evisceration	100 %	Transponder trace stop			
T collection		100.0 %			
T free in abdomen cavity		75.0 %			
T enveloped in omentum fat		17.3 %			
T extraperitoneal site		7.7 %			

The readability of the transponders across each of the different 6 steps of the slaughtering chain was 100% and didn't cause any delay of the operator's work, until the evisceration. The identification of each carcass by eartag stops earlier (after washing, 4th step) than transponder's (at evisceration, 6th step), since eartags were removed not to get burnt at singeing step. Even if the recovery of transponders was 100%, a delay of the operator at

evisceration activity occurred. Such delay was almost due to the 75% of free transponder in abdomen that needed to be picked up because found laying on the diaphragm muscle, beneath viscera, and above all to the 7.7% of extraperitoneal transponders (the hardest to find for the operator). The resting 17.3% were the easiest to recover, since immediately appeared to the operator embedded in omentum fat, after cutting the abdomen at evisceration step. On the whole, the recovery of transponders from the carcass caused a delay of the slaughtering chain times that shifted from 60 to 30 heads/hour. As far as carcass averaged weight and warm yield no statistical difference occurred between the experimental groups.

The auricle tissue matrix resulted to fit the experimental needs as it was suitable for *in vivo* and *post mortem* sampling. Also the muscle tissue was easily sampled from the carcass in the freezing chamber. The DNA amplification by means of 6 microsatellites allowed to distinguish univocally all of the pigs by their genetic profiles. No cases of non identity between the *in vivo* and *post mortem* samples occurred. Moreover, genetic profiles obtained from 11 carcasses from T group meat sampled in the freezing chamber matched with genetic profiles from the respective auricles samples, while muscles' genetic profiles from 7 carcasses from C group showed genetic non identity, as expected, thus revealing the impossibility of passing anonymous carcasses off as labelled carcasses of the EID circuit.

CONCLUSIONS – This trial confirms in 203d old pigs the previous results of the integrated system EID+DNA obtained in 28d old piglets and highlights a higher reliability and accuracy of the electronic identification than ear tags for traceability needs for *in vivo* swine meat chain. Nevertheless, the EID system showed in this trial remarkable limits *post mortem* concerning the recovery of free in abdomen and extraperitoneal transponders. The first outcome due to this difficulty of transponders recovering conditions is represented by a severe delay of the usual time of work, practicly doubled in our slaughtering chain.

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