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Genetic Traceability of livestock products

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ABSTRACT

Aim of this review was to describe the novel approaches on genetic traceability of livestock products. The term traceability, regarding the livestock production sector, means the ability to keep under unfailing control the products origin and animals identity along all passages of the food chain, from farm to fork. In this way it represents a warranty both for the consumers and the producer and it will permit to know where, who and how a product has been produced. It is clear that traceability could be an important tool in order to preserve and to turn to account the livestock products, especially for typical ones. Different kinds of traceability (conventional, aromatic, geographic and genetic) are discussed in order to explain the principles on which they are based and their possible applications. Genetic traceability is based on DNA identification technology through the use of molecular markers. The genetic traceability might be used at four different levels: individual, cohort/group, breed and species. Regarding genetic traceability, the effective discrimination at level of unique animal identification depends on reducing the probability to find two individual sharing, by chance, the same genetic profile to an acceptable low threshold. For example in a standard proceeding even a two locus test with polymorphic markers as microsatellites (one in sixty-four chance of error, i.e. accidental match) might be sufficient to reach a verdict, but for a forensic case, eight loci (one in 16.8 million chance of error) might be sufficient to reach a verdict. The effective discrimination from the point of animals group (herd, breed or species) identification is based on two different approaches: deterministic and probabilistic. Deterministic approach is based on the analyses of neutral molecular markers specific for each breed and/or genes with different allelic forms fixed within breed as genes affecting coat colour. Probabilistic approaches are based on two methods, the first using the allelic frequencies typical of each group (herd, breed or species) while the second using genetic distances among groups. In conclusion, this review, showed as the novel approaches on genetic traceability of livestock products is an available method even if it should be improved in terms of cost reduction for single sample, work effort, reproducibility and accuracy of results. At the time genetic traceability is an important method for origin identification of livestock products and a tool for guarantee conventional traceability system as routine method for food safety.
(Keywords: genetic traceability, DNA identification, livestock products, food safety)

INTRODUCTION

Currently traceability of food production is a priority on the international agenda of various global organisations such as the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO). It estimated that the world population exceeds 6 billion people, who in turn support, and are supported, by some 17 billion poultry and nearly 4.5 billion livestock, according to the latest FAO statistics. With the markets globalisation agriculture has become "anonymous", alimentary raw materials are

produced where they are cheaper and consumers do not know neither about food origin nor the producing companies (*McKean*, 2001). In the last few years the discussion on the identification and registration of GMOs (genetically modified organism), between the EU and the USA, contributed to increase the traceability requirements and transparency in food chains. Labelling of GMOs is obligatory in the USA only if the product differs essentially from the "original", e.g. if the nutritional value differs, or if the product contains an allergen that it is not present in the original. The EU demands that all GMO products, with a GMO contamination of >0.9%, must be labelled as such. Moreover, the social and economical changes in developing countries have focused more attention on the consumer point of view on the origin and food safety of animal products, particularly after negative events such as BSE (bovine spongiform encephalopathy), chicken dioxin contamination and the recent avian influenza that are only some examples which reported attention on animal products traceability and suggested EU legislators to introduce specific new laws (EU regulation 178/2002) in the food safety sector. Therefore, the traceability become an answer of the producers to the consumers that need more public trust in term of safety and quality for food of animal origin. Moreover, in the last years, the valorisation of traditional and protected products, whose Italy is leader in Europe and in the world with 145 PDO and PGI and more than 1400 traditional products (included in the list filled according to the DM 350/1999), is an interesting way for the development of livestock systems located in less competitive areas but that still have to face the market competition. These products embody typical added values represented by tradition, high quality and, sometimes, a close link with animal breeds at risk of extinction resource, allowing their selling at a higher price. Traceability could be a method to safeguard and guarantee the origin of these products as well. Traceability is also considered a fundamental tool in the White Book written by the European Union Commission that represents a basic element of European Regulation concerning responsibility for damages due to "defective" product. Therefore, traceability methods can become an effective way to develop new relations between the world of production and the world of consume.

TRACEABILITY SYSTEMS

The basic characteristics of traceability systems are similar, requiring product identification, product tracking and the maintenance of information relating to the product and its movement. Yet there remains a lack of clear consensus to how traceability is achieved in practice. The ISO 8402 standard defines traceability as "the capacity for establishing a product's origin process history, use and provenance by reference to written records' (*ISO*, 1994). However, like other traceability definitions, ISO 8402 does not define which parameters have to be measured or how history or origin should be determined. In a report on traceability systems, *Golan et al.* (2004) underline three key parameters that can be used to characterise traceability systems, as follows: 1) the breadth; 2) the depth and 3) the precision of the system. The breadth of the traceability system is due to the amount of information recorded (e.g. feed regime, pedigree information or details of animal's veterinary care), the depth of the system is how far back or forward the system tracks (to a grain elevator, farm or field); in many cases, the depth of a traceability system is determined by its breadth or attributes of interest. The precision of the system is the degree of assurance with which the tracing system can pinpoint the movement of a particular product, and is described with reference to an acceptable error rate, or what would happen if there were mistakes in tracking the product.

An important key of any traceability system is the ability to clearly identify what it has to be traced. Ideally the product identifier should uniquely guarantee that the identification of the unit or batch is sure (fraud proof), permanent, retaining identity throughout the product life-cycle, simple to read and capture identifying data and not hinder its host.

In practice no single identification system is likely to meet all these requirements, for this reason the choice of method(s) will ultimately be determined by the specific needs of the supply chain in question.

In general terms, at present, there are three different types of traceability available: a) conventional, based on labelling of food (the present law on labelling of beef meat is a clear example); b) aromatic or geographical, based on identification of specific aromatic compounds and on the presence of specific micro organisms in typical products of specific areas; and c) genetic, based on DNA analysis.

CONVENTIONAL TRACEABILITY METHOD

The conventional traceability method is based on external identifiers that are applied to the animal/product and can become unwieldy to implement in more complex supply chains. External identifiers types include both manual methods such as paper labels, in brands (tattoos) and plastic ear tags, and electronic methods such as Radio Frequency Identification (RFID) tags and inject able microchips. The advantage of these approaches lies in they ability to encode different types of information (barcode symbologies can contain information relating to the product and its process history), and the relative ease with which the data can be read in real-time, facilitating the use of electronic identifiers for monitoring animal movements. For example, maintaining individual animal traceability within a meat processing environment could lead to a proliferation of labels to track all the pieces of an animal post slaughter. But possible of greater concern is the fact that external identifiers may become separated from the product through tag/label loss or removal, and are susceptible to fraud. Within the meat processing sector, an EU report found that through the use of a conventional meat labelling system "in many member states serious deficiencies were found in the ability to trace back meat from retail and distribution centres, even to the preceding stage of the production chain" (*European Commission*, 2003).

AROMATIC AND GEOGRAPHICAL TRACEABILITY

The aroma concept is based on the sensory characteristics tied to the presence of volatile substances (of low molecular weight) and not volatile substances (of higher molecular weight) present in animal products (milk, meat, cheese etc.). Some factors that modify the aroma are the heat treatments before the packaging, the cycle oestral of the animal and the type of feeding; it seems in fact that the milk ones produced in mountain area has a more intense aroma which depends on the diet of the bovine to the pasture. In order to verify the effect of herd management influences on aromatic property of milk, *Bailoni* and *Mantovani* (2000), compared the value of some compounds determining the aroma in dairy herds of the plan fed with traditional feeding or unified with dairy herds fed with fresh mountain forage; in the second group they detected a significant increment on aldehydes and ketones.

The geographic traceability instead is based on the determination of the geographic origin studying the bacterial composition of natural serum cultures for the production of cheese products. In fact such coltures could introduce some differences in the products

microflora due to the various area of production, therefore they would allow to distinguish cheeses, even of the same variety, but produced in different geographic areas. An example has been put to point for the mozzarella in the area of Caserta and Salerno (*Mauriello et al*., 2003).

GENETIC TRACEABILITY

Genetic traceability system might be considered as a biometric labelling system that incorporate biological data and cannot easily be faked, altered or appropriated. This biotechnology includes DNA profiling, retinal scanning and nose printing. Moreover, to being less prone to error or fraud, these biometric labelling methods are permanents, covering the life history of the animal, and in the case of DNA the full product life history. The basic principle underlying DNA-based traceability is that each animal is genetically unique (except in the case of identical twins or clones) and that the animal's own DNA code can be used to identify it and its products as its own label. *Jeffreys et al.* (1985) discovered that when DNA is digested with specific enzymes, the pattern of resultant DNA fragments, resolved by gel-electrophoresis, is specific to the individual. This process became widely-known as DNA fingerprinting. This technology was initially applied in forensic studies and proved an extremely powerful source of evidence in legal cases. However DNA fingerprinting required a relatively large amount of high quality source DNA and this was not always available, particularly in forensic cases. The development of another process by *Mullis et al*., (1986), and the application of this process to a particular type of DNA sequence led, in 1989, to the development of current DNA identification technology. The basic principle was to generate, in a test tube, large quantities of specific target DNA sequences, where the sequences are specifies by a pair of short (around 20 bp) artificial DNA primers. This process, which is known as the polymerase chain reaction (PCR), has become the pillar (foundation) of modern molecular genetics. However, the PCR alone is not sufficient to allow individual identification. It is necessary to find sequences of DNA that vary among individuals. In 1989, the PCR process was first applied to a type of variable DNA sequence called simple tandem repeats (STR) or microsatellites, and this led to present day genetic identification more generally known as DNA profiling.

LEVELS OF GENETIC TRACEABILITY

The genetic traceability might be used at four different levels: individual, cohort/group, breed, species.

Individual traceability is a food safety control able to guarantee the consumers from frauds, it is of a great importance in the beef sector as control of the conventional labelling system (*Portetelle et al*., 2000; *Sancristobal-Gaudy et al*., 2000; *Cunningham et al*., 2001; *Arana et al*., 2002). However, for the milk-cheese chain and other animal products made by groups of animals the individual traceability is not directly useful but only to reconstitute the origin group or cohort. This system might be very interesting for cheese and other products by multi-individuals (*Cocucci et al*., 2002) in this case the right term is herd or cohort traceability.

Breed and species traceability can verify with scientific and objective methods the origin of animal products (*Milanesi et al*., 2003; *Ciampolini et al*., 2006; *Ovilo et al*., 2000; *De Marchi et al*., 2003); it is of particular interest for products such as cheese and processed meat, that are strictly linked with only one breed or species (*Alves et al*., 2002).

In this case traceability is very useful for quality certification as the European PDO and PGI label that can support the economic development of marginal areas increasing the add value of typical or niche products often linked to animal genetic resources under conservation schemes (*Gandini* e *Oldenbroek*, 1999; *Milanesi et al*., 2003). For these reasons breed traceability is an important topic of research in Mediterranean countries (Italy, Spain and France) where it could be found a high number of typical products (*Pancaldi et al*., 2005) that often are mono-breed. In Italy there are some famous PDO "monobreed" cheeses such as the Fontina Valdostana (obtained with milk of the Valdostana cattle breed) and the Parmigiano Reggiano obtained only by the Reggiana cattle breed. There are also other examples of typical monobreed cheeses but, in this case, they are not yet protected by the European label (the Spressa and Morlacco produced by the Rendena and Burlina cattle breeds, respectively). In the beef sector there is also an example of PGI labels (since 1998) of the "Vitellone Bianco dell'Appenino Centrale" made by the Chianina, Marchigiana, Romagnola, Maremmana and Podolica cattle breeds. The pig sector based on ham production it is also interested on traceability method not firstly for identify the breed of origin but for exclude the use of specific pig breeds such as the Pietrain and Belgium Landrace that produce meat of low quality characteristics.

Following the classification proposed by *Ajmone-Marsan et al*. (2004), the studies on breed traceability were based on two different approaches: a) deterministic approach and b) probabilistic approach. The deterministic approach is based on the analyses of neutral molecular markers specific for each breed (*Negrini et al*., 2003; *Alves et al*., 2001) and/or genes with different allelic forms fixed within breed (Miladnesi *et al*., 2003). The major researches on this approach are based on genes affecting coat colour (*Crepaldi et al*., 2003; *Russo* and *Fontanesi*, 2004; *Maudet et al*., 2002; *Carriòn et al*., 2003; *Alderson et al*., 2003; *Fernandez et al*., 2004). The probabilistic approach is based on two methods, the first one using the allelic frequencies typical of each breed, and the second one using the genetic distances among breeds (*Milanesi et al*., 2003).

POWER OF DISCRIMINATION (NON È MEGLIO LEVEL)

Generally, for a traceability system, the basic question is whether two samples are the same or different. The answer, in the case of a genetic traceability method, is a matter of probability. Indeed, if we assume for an individual animal, ten individual simple tandem repeats (STR) loci and each STR have four alleles, for each STR the animal possess two of the four possible alleles, one inherited from the father and one from the mother. The two alleles, together are refereed to as a genotype. Therefore, ten genotypes represent the twenty alleles in the ten loci STR profile for this animal. The probability that any other animal shares, by chance, this exact combination of genotypes is low. For example if the frequency of each of the four alleles for the ten STR is assumed to be 25%, the cumulative probability (%) of a chance match is 9×10^{-8} or $0.125^{n}\times100$, where n is the number of loci considered.

Hence, the effective discrimination to the point of unique animal identification depends on reducing this probability to an acceptably low level. The basic for declaring a match depends on the purpose to which the information is to be put. In a standard proceeding, for example, even a two locus test (one over sixty-four chance of error, i.e. accidental match) might be sufficient to reach a verdict, in a forensic case, eight loci (one in 16.8 million chance of error) might be sufficient to reach a verdict.

A significant new development in the field of molecular genetic identification is currently underway. A new class of DNA markers called single nucleotide

polymorphisms (SNPs) has been researched. As the name indicates, a SNPs concerns genetic variation at the lowest possible level that is at a single base or nucleotide. As a result, the amount of genetic variation in each such unit is limited. In contrast to microsatellites with numerous alleles, SNPs have only two alleles. This makes SNP analysis highly amenable to full automation. A possible limitation is that a larger number must be tested in order to achieve satisfactory power of discrimination.

CONCLUSIONS

The livestock production sector is addressed towards a future in which the herds will be specialized in advantaged areas, instead in the disadvantaged areas will be necessary that the companies are adapted to have a multifunctional role not being in a position to being competitive in terms of production. In both cases the companies will have to guarantee a sustainable development and to find a balanced combination between new technologies and protection of the typical products giving always greater importance to the alimentary emergency and food safety using traceability systems.

Different kinds of traceability systems (conventional, aromatic, geographic and genetic) are available for field application. At present, genetic traceability, based on technology of DNA identification trough the use of molecular markers, seem to be very important for guarantee conventional traceability system as routine method for food safety.

In the next future, the reduction of costs and the organisation system for recording and stocking organic and DNA samples will permit an application of genetic traceability as more routine method even if the reproducibility and repeatability of these molecular methods should be studied.

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REFERENCES

Ajmone Marsan, P., Milanesi, E., Negrini, R. (2004). Tracciabilità di razza con metodi molecolari. Proceedings of 7th World Conference of the Brown Swiss cattle breeders. Verona, 3-7 Marzo, 101-105.

Alderson, G.L.H., Plastow, G.S. (2003). Use of DNA markers to assist with product traceability and pedigree analysis and their role in breed conservation. AGRI. 35. 1-7.

Alves, E., Castellanos, C., Ovilo, C., Siliò, L., Rodriguez, C. (2002). Differentiation of the raw material of the Iberian pig meat industry based on the use of amplified fragment lenght polymorphism. Meat Science. 61. 157-162.

Arana, A., Soret, B., Lasa, I., Alfonso, L. (2002). Meat traceability using DNA markers : application to the beef industry. Meat Science. 61. 367-373.

Bailoni, L., Mantovani, R. (2000). Caratterizzazione dell'aroma del latte bovino prodotto nel Parco Adamello Brenta e possibilità di valorizzazione. Atti del convegno "Formaggio razza Rendeva: un'opportunità per la razza Rendena e la Val Rendena". Caderzone (TN). 27 agosto 2000.

Barua, A., Yoshimura, Y. (1997). Rural poultry keeping in Bangladesh. World's Poultry Science Journal. 53. 387-394.

Carriòn, D., Day, A., Evans, G., Mitsuhashi, T., Archibald, A., Haley, C., Andersson, L., Plastow, G. (2003). The use of MC1R and KIT genotypes for breed characterisation. Arch. Zootec., 52. 237-244.

Ciampolini, R., Cetica, E., Ciani, E., Mozzanti, X., Fossella, F., Marroni, M., Biagetti, C., Sebastiani, P., Papa, G., Filippini, G., Cianci, D., Presciuttini, S. (2006). Statistical analysis of individual assignment tests among four cattle breeds using fifteen STR loci. Journal of Animal Science. 84. 11-19. SSn: 0021-8812.

Cocucci, M., Ferrari, E., Martello, S. (2002). Il latte dall'azienda agricola alla distribuzione: esempio di rintracciabilità di filiera. Industrie Alimentari. 41. 311-316.

Crepaldi, P., Marilli, M., Gorni, C., Meggiolaro, D., Cicogna, M. (2003). Preliminary study on MCR1 polymorphism in some cattle breeds reared in Italy. Italian Journal of Animal Science. 2. 1. 13-15.

Cunningham, E.P., Meghen, C.M. (2001). Biological identification systems: genetic markers. Rev. Sci. Tech. Off. int. Epiz., 20. 2. 491-499.

De Marchi, M., Targhetta, C., Contiero, B., Cassandro, M. (2003). Genetic traceability of chicken breeds. Agriculture Conspectus Scientificus. 68. 4. 255-259.

European Commission (2003). Overview report of a series of missions carried out in all member states during 2002 in order to evaluate the operation of controls over the traceability and labelling of beef and minced beef. General report Dg (SANCO) 9505/2003. Fod and Veterinary office of the health and Consumer protection directorate, European Commission, Brussels, 11.

Fernández, A., Fabuel, E., Alves, E., Rodriguez, C., Silió, L., Óvilo, C. (2004). DNA tests based on coat colour genes for authentication of the raw material of meat products from Iberian pigs. Journal of the Science of Food and Agriculture. 84. 14. 1855-1860.

Gandini, G.C., Oldenbroek, J.K. (1999). Choosing the conservation strategy. Genebanks and the conservation of farm animal genetic resources. Ed. J.K. Oldenbroek, The Neatherlands.

Golan, E., Krissof, B., Kuchler, F., Nelson, K., Price, G. (2004). Traceability in the US food supply: economic theory and industry studies. Agricultural Economic report no. AER830, march. Economic research service of the United States department of Agriculture, Eshington, DC, 56.

International Organization for Standardization (ISO) (1994). ISO 8402: Quality management and quality assurance – vocabulary. ISO, Geneva.

Jeffreys, A.J., Wilson, V., Thein, S.L. (1985). Hypervariable 'minisatellite' regions in human DNA. Nature. 314. 67-73.

Maudet, C., Taberlet, P. (2002). Holstein's milk detection in chesses inferred from melanocortin receptor (MC1R) gene polymorphism. J. Dairy Science. 85. 707-715.

Mauriello, G., Moio, L., Genovese, A., Ercolini, D. (2003). Relationships between flavoring capabilities, bacterial composition, and geographical origin of natural whey cultures used for traditional water-buffalo mozzarella cheese manufacture. J. Dairy Sci., 86. 486-497.

McKean, J.D. (2001). The importance of traceability for public health and consumer protection. Rev. sci. Tech., 20. 363-71.

Milanesi, E., Negrini, R. (2003). Tracciabilità delle razze con diverse strategie di ricerca genetica, con particolare riferimento alla razza Romagnola, Proceedings of the seminary "La razza bovina Romagnola" Bologna 31th January.

Mullis, K., Faloona, F., Scharf, S., Saika, R., Horn, G., Erlich, H. (1986). Specific enzymatic amplification of DANN un vitro: the polymerase chain reaction. Cold Spring Harb. Sym. Quant. Biol., 51. 263-273.

Ovilo, C., Cervera, M.T., Castellanos, C., Martinez-Zapater, J.M. (2000). Characterisation of Iberian pig genotypes using AFLP markers. Animal Genetics. 31. 117-122.

Pancaldi, M., Carboni, E., Paganelli, A., Righini, G., Salvi, A., Fontanesi, L., Russo, V., Davoli, R., Beretti, F., Ravaglia, S. (2005). Come garantire l'autenticità dei prodotti agroalimentari tipici. Utilizzo combinato di traccianti biologici e analisi del DNA. Industrie Alimentari. 44. 1127-1134.

Portetelle, D., Haezebroeck, V., Mortiaux, F., Renaville, R. (2000). Traçabilité dans la filière animale. Biotechnol. Agron. Soc. Environ., 4. 233-240.

Russo, V., Fontanesi, L. (2004). Analisi dei geni del colore del mantello: tracciabilità di razza. Proceedings of 7th World Conference of the Brown Swiss cattle breeders. Verona, 3-7 Marzo, 101-105.

Sancristobal-Gaudy, M., Renand, G., Amigues, Y., Boscher, M.Y., Leveziel, H., Bibé, B. (2000). Traçabilité individuelle des viandes bovines à l'aide de marqueurs génétiques. INRA Prod. Anim., 13. 4. 269-276.

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