Diversity of animal identification techniques: from 'fire age' to 'electronic age'

Gerardo Caja, J.J. Ghirardi, M. Hernández-Jover & D. Garín

Grup de Recerca en Remugants, Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

Abstract

Animal marking is associated to the domestication of different animal species by humans for various reasons. Identification techniques are classified according to characters used and to their permanence on the animal. Main artificial permanent systems are branding (hot-iron and freezing), tattooing, ear notching, ear tagging (metal and plastic) and electronic identification (injectable, ear tags and bolus), but natural systems are also used (mainly retinal imaging and molecular markers). Recently artificial systems and natural systems have been combined as a way to provide a real time tagging and tracing-back methodology for on farm use and administrative purposes until slaughtering, and as a method to audit the tracing-back of animals, carcasses and meat cuts in the food chain.

Keywords: tagging, marking, branding, tattooing, ear tags, electronic identification, transponders.

Introduction

Animals marks have been used by herders since the Neolithic period and are strongly associated to the domestication of animals (Landais, 2001). Different methods of marking animals were used by Egyptians, Greeks, Romans, nomadic people of Scandinavia, Asia and Africa, and pre-Hispanic Americans for different purposes.

Animal identification methods could be classified according to the nature of the characters used (natural or artificial), and to the permanence of the character on the animal (permanent or temporary). Natural characters (e.g. coat color, horns, hair curls, fingerprinting) are generally used for animal recognition, while artificial characters (marks) are made by humans for different purposes. Permanent marks (indelible), are applied as signs of individual identification, ownership or protection (e.g. animals in quarantine); and, temporary marks (e.g. erasable or removable) are useful for animal management.

Many identification techniques are possible in practice due to the large diversity of domestic animal species, breeds, productive purposes, exploitation systems and environmental conditions, Moreover, the large number of identification methods and devices currently used clearly indicates that not one is fully satisfactory. Retention rate values of 95% (two years) to 98% (one year) and 85% readability are considered acceptable in many cases (Stanford *et al*., 2001; ICAR, 2003) but are not fully achieved. Available methods for animal identification have suffered dramatic changes in the last decades, but surprisingly, old and new techniques coexist in most countries.

The global trade of live animals or animal products, has dramatically increased the risks of human and animal disease outbreaks and makes difficult the traceability in the food and feed chains. The European 'White paper on food safety' published in 1999, places traceability as the backbone of all policies concerning food safety. Exporting countries need to be prepared to meet the new traceability requirements of importing countries, and this gives added impetus to animal identification methods which have not been sufficiently supported before such us herd management and improvement, quality monitoring of livestock products, and welfare or health control requirements.

Current animal traceability requires, at least, the use of a unique and individual identity code for each animal, and a transparent, credible and verifiable system to assure identity (McKean, 2001). Two recent European regulations for the identification and registration of cattle and beef (CE 1760/2000) and sheep and goat (CE 21/2004) have specified the identification requirements for animals and meat interchanges in the European Union. Standardized ear tags are currently the approved identification device for cattle (from January 1 of 1998), sheep and goat (from July 9 of 2005), but logistic problems (nearly 300 million of animals in the EU) make the use of electronic identification for automatic animal recording and data management recommendable. For this reason, a decision will be taken in 2006 to make mandatory (from January 1 of 2008), in sheep and goat EU countries with more than 600 000 animals, the use of electronic identification of these species. Moreover, the extension of the electronic identification as an official identification system for cattle in the European Union is also now under study.

Identification techniques

Animal identification methods in history were reviewed by Sánchez Belda (1981) and more recently by Blancou (2001) and Landais (2001), distinguishing between permanent and non permanent systems. Reasons for using a particular identification system vary in history according to the cultural and economical conditions of human societies. Currently, the main reasons for using an animal identification system in the modern livestock industry are:

- to **indicate property ownership, for which** registered brand or marks is used. Permanent marks are the only identification system acceptable by law as a legal proof of identity and ownership.
- to **identify individual animals, as** a prerequisite for individual performance recording in improved breeding and management systems. Temporary systems may also be used as auxiliary for this purpose.
- to allow a mechanism for **disease and residue traceback** to the property of origin. Permanent marks are again the only acceptable identification system for this purpose.

Main systems used for permanent animal identification in the current livestock industry include:

1) branding (by fire or freezing), ear marking (by notching, tattooing and ear tagging);
2) electronic identification (injectable, ear tag, and bolus), and
3) natural characters (DNA genotyping and retinal images); and are here reviewed and compared.

Traditional identification techniques

Hot iron branding

Hot iron branding causes a scar where the hair regrows in a different pattern than on the surrounding skin. Branding of cattle, horses, mules, and buffalo is traditionally done by using a hot iron. Sheep and goat were also branded in the past (normally on the nose or cheek), but less frequently than in cattle or horses. This ancient method of marking is forbidden in countries with advanced animal welfare laws (DEFRA, 2003), but is still an official sign of ownership in many countries where books of marks are currently operative for cattle and horses. More humane options for marking animals are readily available and this method should only be used when other methods are not possible.

A single letter, numbers, simple figures or bars are normally not accepted as ownership branding marks for most livestock, but more than three letters is not recommended (NDA, 1966). Different types of irons are used, from the most simple (bars, letters or single numbers from 0 to 8) to the most elaborated (forged symbol of the owner) and uses include identification of property (e.g. owner initials or symbol) and individual marks (e.g. year of birth and serial number). When composed letter or numbers are branded, separate irons for each character are preferred and a distance of 10-12 cm must be maintained. Branding irons are usually made of mild steel alloys, which are generally better heat conductors than mild steel. The surface of the branding iron should be flat, smooth, and no more than 4 mm wide, and an iron handle of 45-60 cm long is also recommended.

Calves and foals are usually branded before weaning when they are 3-5 months old. At this age the hide is thick and the animals are easier to handle or restrain. A calf-marking cradle may be used for easier and safer restrain during branding (Figure 1). If animals are branded when they are too young, the brand grows with the hide and will greatly reduce hide value at an adult age.

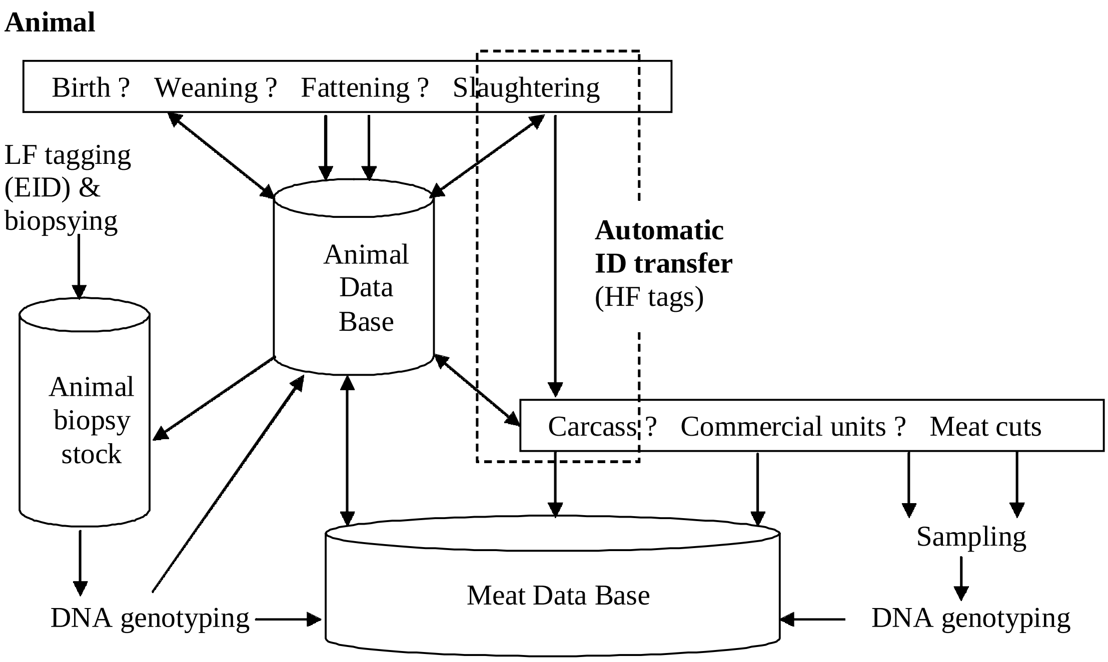

Figure 1. Combining electronic identification (EID) and DNA fingerprinting for traceability of animals and meat in the meat chain: Information flow.

Iron application should be performed when iron is hot-white or ash-grey colored and not exerting excessive pressure, allowing the iron to do the branding. Recommended procedure (Hurst and Irwin, 2000) is to put the iron against the animal's skin for 5-6 s (3 counts), without pressure, and roll the iron with the shape of the animal's body to apply the same pressure at all points of iron contact. Long application, overheating, rough use or damage to the branding surface of the iron will cause incorrect brands. Thereafter, cold water or wound oil should be sprayed on the mark to reduce burning effects and to improve healing. For long-coated cattle, the branding area should first be clipped.

The adequate method of heating the irons is a fire of wood or bark burned to coals. Gas burners for heating brands are easily portable and more convenient than traditional wood fires. Coal or coke must never be used, as they burn at too hot a temperature. Electrically heated branders are also available in the market. The correct heat for branding is a blue flame that will instantly burn a piece of paper or board. If the heated iron shows any red, it is too hot. When branding is finished, the hot irons should be cleaned and submerged in sump oil to cool and protect from oxidation. Fire branding should never be performed in rainy weather, or

on hides that are wet, because the hot iron boils any moisture in the coat and scalds the surrounding area. Unnecessary pain to the animal and no regular in shape brands will be caused in these conditions.
Location of the brand on the animal body is chosen for visibility (flank, rump) but alternative locations (cheek, hind limb) are currently recommended to avoid hide depreciation. Light branding (Landais, 2001) or hoof branding (in horses; Sanchez Belda, 1981) are also used for temporary marking of animals intended for sale. In this case the hot iron is lightly and briefly applied to the animal, aiming to burn the coat but not the skin. Branded animals should not be sold for slaughter within 3 weeks of the operation. If sold within the 3 weeks, the purchaser should to be informed in writing.
Nevertheless, hide is a co-product of the cattle meat industry and in most countries the hide makes up 10-15% of the total value of the animal. It is estimated that branding reduces hide values by $10-20 per hide. A brand placed in the correct position (left rump) results in minimum trimming when the hide is processed by the leather industry.

Caustic branding

Caustic chemicals (corrosive acids, caustic soda paste, caustic potash) were proposed as an alternative to hot iron for branding in cattle. Although not using fire is a great advantage in practice, caustic branding is difficult to apply with accuracy and gives irregular results. Moreover, according to the new welfare regulations in many countries (DEFRA, 2003), it is a painful and not recommendable identification method and it should not be used to brand animals.

Freeze branding

A freeze brand may replace an iron brand in dark coated animals, as initially used in dairy cows and most recently also in horses and mules. Advantages of freeze branding, when compared to hot iron branding, are less discomfort and reaction from the animal. Freeze branding is less damaging for hide than fire branding if the application period is adequate, and no weakness occurs in the leather. Disadvantages of freeze branding are that it is more expensive and time consuming than fire branding, the final brand takes up to 4 months and the technique is less suited to light-colored stock. Moreover, freeze brands may be temporary tinted for fraud. Nevertheless, freeze branding is accepted as a reasonable identification method in most of cases (DEFRA, 2003).
The main effect of freeze branding is to destroy the cells that produce the pigment in the skin and hair (melanocytes). After the skin is exposed to the chilled branding iron, it is frozen in the shape of the brand applied and within 2-3 min the skin thaws and the area reddens. A marked edema with fluid-filled swelling develops 5-10 min after brand application, and persists for approximately one day, depending on the exposure time. The edema then recede, and the branded area becomes dry and scurfy. Varying amounts of skin and hair are lost over the next 2-3 weeks. These areas are generally legible until white hair growth

becomes evident. Overexposure to the freeze brand may result in excessive hair follicle loss in the centre of the brand, and consequently the growth of white hair will occur only on the edges of the brand site. Subsequent hair growth occurs usually 6-10 weeks after branding, depending on the season. Freeze branding produces a permanent mark on the skin, the hair re-growing in a lighter colour and the skin itself lacking in pigments. The resulting brand, if adequate, is legible from about 30 m. In the case of white and grey horses a bald area is frequently observed after freeze branding.

Although liquid N was used initially, it is expensive and more care is needed by the operator. The temperature of liquid N is lower, and the application timing is much more critical in order to avoid overfreezing the brand. Moreover, it can only be transported in suitable thermos with vented tops. Dry-ice made directly from a CO_2 cylinder or dry ice-methyl alcohol mixtures are more currently used than liquid N. For application, clipping the brand site as close to the skin as possible and removing loose hair and dirt, which increases time and preparation requirements is recommended. Soaking the brand site with methylated spirits immediately before applying the brand, and repeating for each character improve the brand. The brands moulds are cold enough when bubbling (boiling) stops and application on the hide for approximately 15-40 s depending on freezing solution and age of the animal (Table 1). Restraint of the animal is essential. Animals in poor condition do not brand as well as those in moderate to good condition. The branding of calves (under 4 months) is not recommended.

Freeze branding irons should be made of copper or bronze alloy. Solid copper is the best but it is most expensive. Conventional steel irons work but are more likely to result in a poor unreadable brand. The face of the irons should be rounded to uniformly transfer the cold from the iron to the skin. Suggested dimensions for the branding face are 6-10 mm wide, 70-100 mm high and 38-50 mm deep. Handles should be about 380 mm long. Approximately 7 kg CO_2 will produce enough dry-ice to fill

Table 1. Freeze-branding time for legible brands in cattle and horse.

	Freezing solution	
Animal specie and age	Dry ice (–70°C)	Liquid nitrogen (–197°C)
Cattle:		
Calves 4 to 8 months	25 s	15 s
Yearlings	25-30 s	20-25 s
Adults	35-40 s	25-30 s
Horse:		
Foals	-	6-12 s
Adults	-	8-12 s

approximately 100 digit molds. A mixture of 5 kg dry-ice to 9 l of methylated alcohol is enough for 150 animals to be branded with three characters each. On animals with light-colored coats, a bare (hairless) brand can be made by holding the iron on for 50-60 s, which is longer than is necessary when applying a brand to a dark-coated animal. The brand must remain in the cooler for 60–90 seconds after each use. Branding time should be increased on dark coats and thicker hides. Care should be taken when handling freeze-branding coolers because they can produce frostbite in the human skin.
A new freeze branding system is also available for cattle and horses based on digit moulds which are filled with dry ice on the site by using a special gun and a liquid-withdrawal tank of CO_2. Recommended times for branding are longer (horses, 2 0s; dairy cattle, 60 s; and, beef cattle, 90 s). Branding with this system is more easy and accurate.

Paint branding

Sheep branding is usually done by painting the wool after shearing with the symbol of the owner or with digit moulds similar to those used for freeze branding. As this mark is temporary, a second system (ear notching, tattooing, ear tags, etc..) needs to be used for a permanent indication of ownership. Paint is also used for short term marking of other livestock species (cattle, pigs,…). With this aim sprays of biocompatible paints and wax colored sticks are used.
Life of paint branding is long in fine wool sheep breeds, but it is short and unsatisfactory in coarse wool breeds. Same problem is observed in hairy lambs.
Paint must be washable to avoid wool depreciation. Colors commercially available are usually yellow, blue, green, black, red, or purple. Brands are usually painted for the side, hip, nose, or jaw on either the left or right side of sheep. No owner brand should be recorded across the back of a sheep, which are normally reserved for individual sheep numbers in most countries.

Ear notching

Ear notching is a very old practice in cattle, sheep, goat and pig, as a chip and permanent system to indicate ownership. They are made by knife cutting or by using special cutting pliers. Old Spanish flock books (De la Maza, 17XX) included detailed information on paint branding and ear notching of sheep.
Ear notching is worldwide used for holding identification and in some cases as a cheap system for numbering. Moreover, tuberculosis positive cattle was marked in the past with a T notch in the ear to identify animals to be slaughtered. Ear wound necrosis and breakage, as well as development of fly worms on the wounds may alter the notch codes.
A mathematically interesting system of numbering based in ear notches is still being used in pigs (Official Berkshire Ear-Notching System). In this system (Figure 2), a smart combination of notches in the right ear (coded as numbers 1, 3, 9, 27 and 81) and in the left ear (coded as numbers

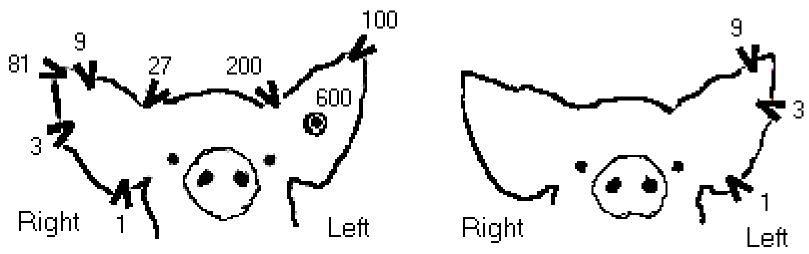

Figure 2. A mathematically interesting system of numbering based in ear notches is still being used in pigs (Official Berkshire Ear-Notching System).

100, 200 and 600), are used for litter marking. Up to 1 199 litters can be marked with this coding system. Right ear is also used to add the individual marking of a pig inside a litter (coded as digits 1, 3 and 9). Each pig in a litter will have the same notches in the right ear and different notches in the left ear.

Tattooing

Ear tattooing is one of the best conventional methods of permanently identifying animals. The number code that is applied will be in most cases permanent throughout the animal's life. Tattoos are usually applied on either the left or right ears (all species), lip (horses), groin (pets) and under the tail (sheep and goat). Since the tattoo can only be read when the animal's head is restrained, it should be used in conjunction with another system which allows the animal to be identified in the paddock. Black dye paste is normally used for tattoos, but green dyes are preferred with dark or black-eared breeds. The use of a back light may also help to read tattoos in animals with dark skin.

Tattooing should be done with restrained animals in an skin area which is free of hair, cartilaginous ridges and large veins. Tattoos in the top half of the ear retain their clarity better than those in the bottom half. Although ear is the most common place for tattoos, horses were widely tattooed in lower lip in the army and in many purebreds. For dairy sheep and goat the base of the tail was also commonly used. In both cases it is recommendable to tattoo the numbers towards down for easy reading. Tattooing hammers with big numbers were also used for tattooing the holding numbers in pig expedition, although this practice is not recommended currently because is increasing pig stress at transportation to slaughtering.

For better tattooing, skin should be cleaned and wax in excess removed by using alcohol. After cleaning and applying the dying paste on the area to be tattooed, the tattooing pliers should be applied firmly and

quickly, making sure the digits are the right way up for reading. Thereafter, he tattooing paste should be rubbed strongly into the punctures.
Brass digit plates with nickel plated steel needles punch out clean and clear tattoos. Commercially available digits for tattooing range between 5 and 20 mm high. The minimum ear tattoo size for lambs, kids and piglets is 10 mm; for calves, sheep, goat and pigs is 15 mm, and larger numerals should be used for adult cattle. Two sets of numerals are necessary if both young and adults are to be tattooed in a farm. Rotary 4-chain tattoo devices are also available for marking large number of animals. Carefully disinfection of the tattooing digits is recommended to avoid infections and diseases transmission. Moreover, frequent ear tissue necrosis or fly worm attacks are described after ear tattooing in subtropical conditions (Garín *et al.*, 2003).

Conventional ear tags

Ear tags are currently the most common method of identifying individual animals in practice. They can be done in a great variety of shapes (flag, button, loop, etc…), materials (metal and plastic), sizes and colors. Only tamper-proof and non reusable ear tags should be considered as a permanent means of identification. Ear tags are easier to read if numbered with the same numbers on both sides, which is recommended for practice. Retention rate of ear tags is extremely variable ranging from 60-98% depending on tag features, species, breeds and environmental conditions. Nevertheless, little information is available in scientific literature and biocompatibility of most materials is questioned. Animal welfare in regard to ear tags is also questioned in some breeds and conditions.
Resistance to environmental conditions and biocompatibility are critical features for choosing the materials used in ear tags. The placement site is specific for each type of tags and critical for its permanency on the animal. Moreover, environmental conditions affect infection rate of newly applied ear tags, and no ear tagging is recommended with very hot temperatures or during fly activity season. It is advisable to perforate the ear one or two weeks before application in order to reduce the risk of infection of the tagging site. Dipping tags in an antiseptic solution before application is a controversial practice but it is thought that it helps to improve retention and to reduce the risk of infection of ear tags.

Metal tags

Metal loop ear tags are made in brass or aluminum. Brass ear tags with tamperproof closing system have been commonly used for cattle tuberculosis and brucellosis control in many countries. Small aluminum loop tags are easy to stamp and to apply, but also easier to remove. Both metal era tags should be placed in the top of the ear, with an overhang of 5-8 mm, and within the inner half of the ear. Placing the tag in the inner portion of the ear means that they are less likely to be torn out.
These tags are very difficult to read unless the animal is firmly restrained but their loss rate is normally very low in most farming conditions.

Small metal ear tags called 'self-piercing' (applied without pliers) have been very used as short term identification devices in the past, but are not currently recommendable because they are easily removed.

Flexible plastic tags

Plastic ear tags are currently the most common method of identifying individual animals in many countries, and when they are well designed and adapted for the animal specie and breed, they are a recommendable option for livestock identification. They are ideal as a management tool but only tamper-proof and no reusable ear tags should be considered for permanent identification.

With developments in plastic industry, plastic ear tags have improved considerably, with free-swinging, soft, self-piercing multicolored types available. Among the different variety of shapes, sizes and colors available, only soft polyurethane ear tags are currently recommendable for greater retention on the animal. Improved metal or hard plastic points for the ear tag pins are also recommended. Plastic ear tags are available pre-numbered or plain. Specific numbers can be mechanically recorded or hand written on the plain tags by using special markers. Laser recording or the use of fluid plastic is recommendable for the permanent recording of plastic ear tags. Addition of bar codes in ear tags is a useful tool for automatic reading and code recording, but the utility of this method is restricted to new ear tags. Less than 20% of bar coded ear tags were successfully read in feedlot calves by Ghirardi *et al.* approximately 6 months after application.

A study of ear structure shows that the ear cartilage is separated by two prominent structures running parallel to each other. It is important for the plastic ear tag be placed in a central position between these ridges, in the proximal half of the ear, and in a place clear of hair. Specific and well designed pliers need to be used at application.

Electronic identification

Electronic animal identification is currently based on the use of radio frequency waves in the low frequency band. This allows the animal tissues to be penetrated with few radiating effects. The EID device is called 'transponder' (from the words transmitter and responder) and passive technology (without batteries) is used in practice.

The passive transponder is a miniaturized electronic radio-frequency device consisting of an integrated circuit (microchip) and an antenna, which is all enclosed in a water-proof protector. The transponder is activated by a signal transmitted by a readout unit called 'transceiver' (from the words transmitter and receiver). The transponder reacts to this signal by emitting an 'information telegram' previously recorded in its memory. Communication between transponder and transceiver can be made by using alternative (half-duplex, HDX) or simultaneous (full-duplex, FDX) transmission. With HDX, the transponder includes a capacitor for increasing energy storage during activation. After transmission of the information telegram and discharge of stored energy, the transponder is dormant until the next activation cycle.

The information telegram is a digital string in which the bits are partitioned in functional segments corresponding to: header, ID code, cyclic redundancy check error detector, trailer and control. An ISO standard (ISO 11784) was approved in 1996 for the ID code of read only (R/O) transponders intended for animal ID. The standardized ID code is a unique 64 bit combination, in which 10 bits correspond to the country code (translatable to a 4 digit number according to ISO 3166 standard) and 38 bits to the animal ID code (translatable to a 12 digit number); the rest (16 bits) are for reserve. Nearly 275,000 million (2^{38}) different ID codes can be programmed according to this standard. A discretional use of 8 bits from the reserve, linked to the country code, for a re-tagging counter (3 bits) and an animal specie indicator (5 bits) were authorized in 2004. The country code can be replaced by a 3 digit manufacturer code given by the ICAR (International Committee for Animal Recording).

The ISO 11785 standard on technical concepts of EID for animal ID, also approved in 1996, recognizes the HDX and the FDX-variant B methodologies for the interchange of information and states the characteristics of transponders and transceivers for a full reading compatibility. Thus, activation frequency was standardized to 134.2 kHz and the length of the transponders information telegram varies according the technology (HDX, 112 bits; and, FDX, 128 bits). A list of manufacturer codes and current ISO complying ID devices is available on the web (*www.icar.org/animal.htm*). Three main types of transponders for animal ID are recognized by ICAR (2003) and are:

- Injectable transponders: For all animal species. The transponders are covered by a bio-compatible glass capsule and are implantable through a needle. They are injected subcutaneously in different body sites.
- Electronic ear-tags: For almost all livestock species. Transponders are included in a plastic round button-tag and used as the female of a conventional plastic ear-tag.
- Bolus transponders: For ruminants only. Transponders are placed into a high specific gravity capsule (bolus) and orally applied to ruminants. They are retained in the fore-stomachs, mainly in the reticulum.

ICAR (2003) also suggests a minimum retention rate of 98% to approve their use as official ID devices in animals.

Since the first international symposium organized by the General Directorate of Agriculture of the European Commission in 1990 (Lambooij, 1991), significant research has been done in Europe on the use of different types of transponders for animal ID. Previous research projects on EID of farm animals granted by the European Commission are: FEOGA CCAM-93-342 (1993-94; Caja *et al*., 1994), AIR3-2304 (1995-97; Geers *et al.*, 1998) and the large scale implementation IDEA project conducted in 6 countries (France, Germany, Italy, Netherlands,

Portugal and Spain) on nearly 1 million animals (1998-2001; Ribó *et al*., 2001). Moreover, several projects complemented the IDEA results in cattle (Fallon *et al*., 2002) and goats (Pinna *et al*., 2003).
Many results related to the use of injectable transponders in ruminants (Caja *et al*., 1998; Lamboij *et al*., 1999; Klindworth *et al*., 1999; Conill *et al*., 2000, 2002) and boluses (Caja *et al*., 1999; Lamboij *et al*., 1999; Fallon, 2001; Garín *et al*., 2003) have already been published, but only preliminary or partial results have been published so far on the IDEA Project (Ribó *et al*., 2001; San Miguel *et al*., 2004). The final report of the IDEA project is currently available on the web (*www.idea.jrc.it/pages%20idea/final%20report.htm*).
Conclusions of the projects showed that efficiency of EID vary with type and brand of the ID device, but efficiency is greater than with conventional tagging systems (metal or plastic ear tags, collars and tattoos) and above the ICAR recommendation (>98%) when certified devices are used. Subcutaneously injected transponders are currently not recommended in ruminants because of the recovery difficulties and risks to the food chain. There is little information on the efficiency of conventional and electronic ear tags in ruminants and results have shown a wide range of variation in losses (2-48%; Conill *et al*., 2000, 2002; Curtis, 2002).
Despite the importance of pigs for the meat industry, few and contradictory results are available on the use of injectable transponders (Lamboij *et al*., 1992, 1995; Stärk *et al*., 1998) and electronic ear tags in swine (Stärk *et al*., 1998; Caja *et al*., 2000; Babot *et al*., 2004), indicating the need for new approaches in the use of EID in pigs. The most important research is focused on determining the optimum injection site to warrant the full recovery of transponders in the abattoir, and intraperitoneal injection may well be an interesting option in practice (Caja *et al*., 2000; Babot *et al*., personal communication; Hernández-Jover *et al*., personal communication).

Natural characters identification

Body marks and silhouette identification

Coat and silhouette patterns (spot description) as well as hair details were widely used for horse and cattle identification in the past.
Most recently, Holstein dairy cattle required a photography for the inscription of a cow in the breed's herd book. Digital pictures made this task easier and some stood book and herd books include pictures of the animals in their data base. Nevertheless, coat features are not a useful tool for individual identification in breeds uniformly coated.

Optical identification

Retinal imaging and iris imaging are the current methods used for animal optical identification in practice. Retinal imaging uses the patterns of the retinal blood vessels to produce a unique image of each eye. The vascular eye pattern is unique between twins, clones and even between eyes of the same animal, and collection of retinal data on the slaughter line is also possible. Retinal imaging is preferred in practice because it is

more appropriate than iris imaging because the iris changes through animal life (Golden, 1998). In addition, images of the iris can be difficult to acquire when corneal diseases occur. It is a non-invasive method in which, after immobilization of the head, a photo of the retinal vascular pattern is obtained through the pupil, in only a few seconds, by using a digital camera. Retinal images are easy to obtain, reliable and low cost. Depending on the animal behavior and the farm restraining facilities, images can be collected directly in pens or in a immobilization chute. Images can also be collected while vaccinations or other examinations are given. In slaughterhouses the system is installed in the slaughtering line, prior to head removal, providing the tracking link between the animal and the carcass.
The cost of taking several images per animal is less than the current cost for one electronic ear tag. Moreover it is a competitive system when compared with other available identification and data collection systems. A tamper-proof system has also been developed by Golden (1998) combining encrypted geopositional and time signals with retinal images. Auxiliary data collection equipment (weighing scales, barcode and electronic identification readers, etc…) can also be connected to the optical imaging device.

Fingerprinting

Different types of body marks have been used for livestock fingerprinting according to the specie characteristics. Only nose prints are still being used currently in

Molecular markers

The use of DNA genetic markers as a tool for individual ID is today a well established methodology in human, plant and animal sciences. Genetic fingerprinting relies on the detection and analysis of DNA polymorphisms (changes in the DNA sequence) that can be found in the genome. Each polymorphic region analyzed can be used as a ‘genetic marker’ to differentiate between individuals, and the combined profile of a set of informative markers allows individual ID (except for monozygous twins that are genetically identical).
The DNA can be extracted and the changes in the sequence analyzed using the ‘polymerase chain reaction’ (PCR) from a single cell. The choice of the markers must take into account the sample type, the conservation procedure and the cost of the analysis.
Different markers can be used to obtain DNA fingerprints, but due to their abundance and high informativity (degree of polymorphism) microsatellites are the markers commonly used for genetic ID in domestic animals (Cunningham and Meghen, 2001). Microsatellites or ‘short tandem repeats’ (STR) consist of repeats of a simple sequence of 2 to 5 DNA nucleotides. At any one DNA site (locus), there are usually several different alleles in a population, each identifiable according to the number of repeated units. These alleles can be detected by using ‘primers’ designed from the unique sequence that is located on either side of the microsatellite.

More than 2,000 microsatellites are currently characterized and located in the genetic map of farm animals as published on the web (*www.thearkdb.org*).

The ISAG (International Society of Animal Genetics) has selected a standardized set of microsatellites to be used in the bovine and porcine DNA laboratory comparison tests. A simulation study has shown that a subset of 8 or more microsatellites are enough to achieve individual ID in cattle whatever the population structure of the sampled individuals (Arana *et al*., 2002). Thus, DNA profiling, through the use of a selected subset of microsatellites, can confirm the ID of two specimens at probability levels up to 99.9% and can be used for the verification process and random auditing of the traceability of animals and meat.

The difficulty to fully automate microsatellite genotyping for high throughput analysis of samples has revived interest in new types of genetic markers. The 'single nucleotide polymorphisms' (SNP) are DNA polymorphisms due to single nucleotide substitutions or insertions / deletions. The SNP are biallelic markers and their informativity is consequently lower than microsatellites. However, as a result of their abundance in the genome and simplicity of analysis, they are an interesting alternative for individual ID. SNP with intermediate frequencies are the most useful and a quantity from 30 to 50 will probably be necessary for livestock ID (Heaton *et al*., 2002; Gut *et al*., unpublished).

Genetic traceability of meat using microsatellites has already been demonstrated in beef samples (Meghen *et al*., 1998; San Cristobal-Gaudy *et al*., 2000; Shackell *et al*., 2001) as DNA can be easily recovered from a biological sample at each step of the production chain, including cured or cooked meals (Meyer *et al*., 1994; Wang *et al*., 2000).

Traceability of livestock and meat by using EID and DNA: 'EID+DNA tracing' European project

Combining EID and DNA fingerprinting for traceability, as proposed by Caja (1998), can consequently be used in the meat chain where biological samples from animals can be used to check the ID of carcasses or meat. The EID provides a real time tagging and tracing-back methodology for on farm use and administrative purposes until slaughtering, and the DNA profile is the method to audit the tracing-back of the ID of animals, carcasses and meat cuts.

With this aim, a research and implementation project was designed and granted in the EU FAIR 5th called 'EID+DNA Tracing' (Reference: QLK1-CT-2001-02229) Detailed information on the project is available on the web (*www.uab.es/tracing*). The project involves 10 partners from 5 EU countries (Germany, Italy, France, Spain and United Kingdom) for 3 years.

The first part of the project is related to the study of animal EID by using bolus (ruminants) and injectable (pigs) ISO complying transponders. An automatic system for the transfer of the animal ID to the carcass based on the use of flexible radio frequency label transponders at high frequency (13.56 MHz) currently used for item management, is being implemented. The project also includes the development of new equipment for on farm

Table 2. Performance comparison of different livestock identification systems on farm conditions.

Identification system	On farm traits						
	Species and breeds	Animal welfare	Cost of devices	Expertise required	Lifespan retention	Reading ability	Tamper-proof
Branding:							
Hot	All	Low	Cheap	Medium	Long	Medium	Yes
Caustic	Some	Low	Cheap	Low	Long	Medium	Yes
Freezing	Some	Medium	Medium	Medium	Long	Easy	No
Paint	Some	Good	Cheap	Low	Short	Easy	No
Ear notching	All	Low	Cheap	Low	Long	Medium	No
Tattooing	All	Medium	Cheap	Medium	Long	Medium	No
Ear tagging:							
Metal	All	Medium	Cheap	Low	Medium	Medium	No
Plastic	All	Medium	Medium	Low	Medium	Medium	No
Electronic:							
Injects	All	Medium	Expensive	High	Long	Easy	Yes
Ear tags	All	Medium	Expensive	Low	Medium	Easy	No
Bolus	Some	Good	Expensive	Medium	Long	Easy	Yes
Imaging:							
Pictures	Some	Good	Medium	Low	Long	Easy	Yes
Iris	All	Good	Medium	High	Medium	Medium	Yes
Retinal	All	Good	Medium	High	Long	Medium	Yes
Fingerprinting:							
Body marks	Some	Good	Cheap	Medium	Long	Easy	Yes
DNA	All	Good	Expensive	High	Long	Difficult	Yes

Table 3. Performance comparison of different livestock identification systems on slaughterhouse conditions.

Identification system	Slaughterhouse traits						
	Official use	Process automation	Reading ability	On line retention	On line recovery	Carcass retagging	Individual traceability
Branding:							
Hot	Yes	No	Medium	No	No	Yes	Poor
Caustic	No	No	Medium	No	No	Yes	Poor
Freezing	Yes	No	Easy	No	No	Yes	Poor
Paint	Yes	No	Easy	No	No	Yes	No
Ear notching	Yes	No	Medium	Medium	No	Yes	Poor
Tattooing	Yes	No	Medium	Medium	No	Yes	Medium
Ear tagging:							
Metal	Yes	No	Medium	Low	Easy	Yes	Medium
Plastic	Yes	Medium	Medium	Low	Easy	Yes	Medium
Electronic:							
Injects	Yes	Yes	Easy	High	Difficult	Yes	High
Ear tags	Yes	Yes	Easy	Low	Easy	Yes´	Medium
Bolus	Yes	Yes	Easy	No	Easy	Yes	High
Imaging:							
Pictures	No	No	No	No	No	Yes	No
Iris	No	No	Medium	High	No	Yes	Medium
Retinal	No	No	Medium	High	No	Yes	High
Fingerprinting:							
Body marks	Yes	No	No	No	No	Yes	No
DNA	Yes	No	Difficult	High	No	No	High

reading and for automatic retrieving of transponders in the abattoir. The tagging devices and new developed equipment is being tested under specific laboratory protocols to warrant its utility under farm and abattoir conditions.

The second part of the project comprises of the study of methods for sampling and analysis of biological samples for DNA fingerprinting of cattle and pig. The DNA samples are used to audit the EID traceability under practical conditions. Data from EID and DNA profiling is processed, coded and stored in a newly developed data base provided with the necessary tools for data comparison and data retrieval.

The third part of the project is the implementation and validation of the whole traceability system (Figure 1) for beef and pork. Two production systems are considered for beef cattle (7,500 calves): 'red' (grazing steers) and 'pink' (baby-beef calves) beef; and for pigs (9,000 piglets): 'white' (crossbreeds in intensive) and 'black' (Iberian in extensive) pork. Additionally 2,000 lambs will also be traced**.**

Finally, the project includes an evaluation of the costs and a cost-benefit analysis of the traceability system in cattle, sheep and pigs under EU conditions. Estimated annual costs of using EID range between 4-10 • / animal with an extra cost of nearly 2 • when DNA sampling is included. DNA analysis cost varies between 5-15 • according to procedures, but less than 5% of samples need to be analyzed for auditing traceability. Mixed strategies are also interesting for animals slaughtered at young ages and they reduce costs. The cost-benefit analysis will determine the profitability of the EID+DNA system over an extended period of 10 years.

Animal data is collected electronically and is being automatically transferred first to a partner data base and then to a central data base for final checking and processing. Moreover, an important objective of the project is to achieve a widespread acceptance of the developed system from producers and consumers. Therefore, the methodology and results of the project will be disseminated to ensure that national authorities and international organizations are kept fully informed.

References

Arana A., Soret B., Lasa I. & Alfonso L. 2002. Meat Sci., 61: 367-373.

Blancou J. 2001. Rev. sci. tech. Off. int. Epiz., 20: 420-425.

Caja G. 1998. Seminario internacional de identificación animal permanente y trazabilidad: 'Del campo al plato'. Senasa-OIE, Buenos Aires, Argentina, pp. 122-124.

Caja G. *et al.* 1994. Contract CCAM-93-342: Final report. FEOGA (Unit VI-G-4), D.G. Agriculture, European Commission, Brussels.

Caja G., Ribó O. & Nehring R. 1998. Livest. Prod. Sci., 55: 279-289.

Caja G., Conill C., Nehring R. & Ribó O. 1999. Comput. Electr. Agric., 24: 45-63.

Caja G., Nehring R. & Conill C. 2000. Meat Automation, 1: 19-21.

Caja G., Vilaseca J.F. & Korn C. 2002. U.S. Patent No. 6,474,263 B2. Nov. 5, 2002.

Conill C., Caja G., Nehring R. & Ribó O. 2000. J. Anim. Sci., 78: 3001-3009.

Conill C., Caja G., Nehring R. & Ribó O. 2001. J. Anim. Sci., 80: 919-925.

Cunningham E.P. & Meghen C. 2001. Rev. sci. tech. Off. int. Epiz., 20: 491-499.

Curtis M. 2002. Farm Weekly interactive, 28 June 2002, 2 pp.

DEFRA. 2003. Code of recommandations for the welfare of livestock : Cattle. Defra publications. London.

Fallon R.J. 2001. Rev. sci. tech. Off. int. Epiz., 20: 480-490.

Fallon R.J., Rogers P.A.M. & Early B. 2002. Electronic animal identification. ARMIS No 4623. Beef Production Series 46, 54 pp.

Garín D., Caja G. & Bocquier F. 2003. J. Anim. Sci., 81: 879-884.

Geers *et al.* 1998. Contract AIR3-2304: Final report. D.G. Research, European Commission, Brussels.

Heaton M.P., Harhay G.P., Bennett G.L., Stone R.T., Grosse W.M., Casas E., Keele J.W., Smith T.P.,Chitko-McKown C.G. & Laegreid W.W. 2002. Mammal. Genome, 13: 272-281.

Hurst R. & Irwin J. 2000. Methods of cattle identification. Agfact A0.2.5, first edition, New South Wales Department of Agriculture, Australia.

ICAR. 2003. ICAR International agreement of recording practices. Section 1.1, Appendix A, Rome.

Klindworth M., Wendl G., Klindworth K. & Pirkelmann H. 1999. Comput. Electr. Agric., 24: 65-79.

Lambooij E. 1991. Automatic electronic identification systems for farm animals. Commission of the European Communities. Serie: Agriculture. Report EUR 13198. Brussels.

Lambooij E., de Groot P.H.S., Molenbeek R.F. & Gruys E. 1992. Vet. Quart., 14: 145-147.

Lambooij E., Van't Klooster C.E., Rossing W., Smits A.C. & Pieterse C. 1999. Comput. Electr. Agric., 24: 81-90.

Landais E. 2001. Rev. sci. tech. Off. int. Epiz., 20: 463-479.

McKean J.D. 2001. Rev. sci. tech. Off. int. Epiz., 20: 363-371.

Meghen C.N., Scott C.S., Bradley D.G., MacHugh D.E., Loftus R.T. & Cunningham E.P. 1998. Anim. Genet., 29 (Suppl. 1): 48-49.

Meyer R., Candrian U. & Luthy J. 1994. J. AOAC Int., 77: 617-622.

NDA (National Department of Agriculture). 1966. Legal identification marks, 1/1996. Info Pak, Resource Centre, Pretoria, South Africa, 4 pp.

Pinna W., Sedda P., Moniello G. & Ribó O. 2004. Small Rumin. Res. (in press).

Ribó O., Korn C., Meloni U., Cropper M., De Winne P. & Cuypers M. 2001. Rev. sci. tech. Off. int. Epiz., 20: 426-436.

Sánchez Belda, A. 1981. Identificación animal. Ministerio de Agricultura, Publicaciones de Extensión Agraria, Manuales técnicos, Serie B, número 11. Madrid, 286 pp.

San Cristobal-Gaudy M., Renard G., Amigues Y., Boscher M.Y., Leveziel H. & Bibé B. 2000. INRA Prod. Anim, 13: 269-276.

San Miguel O., Caja G., Almansa V., Miranda F. & Lueso J. 2004. 34th ICAR meeting, Sousse, Tunisia (in press).

Shackell G.H., Tate M.L. & Anderson R.M. 2001. Proc. Assoc. Advmt. Anim. Breed. Genet., 14: 533-536.

Stärk K.D.C., Morris R.S., Pfeiffer D.U. 1998. Livest. Prod. Sci. 53:143-152

Stanford,K., Stitt J., Kellar J.A. & McAllister T.A. 2001. Rev. sci. tech. Off. int. Epiz., 20: 510-522.

The European Community, Caja G., Vilaseca J.F. & Korn C. 1998. Patent P.T.C. Pub. No. WO98/01025. Jan. 15.

Wang R.F., Myers M.J., Campbell W., Cao W.W., Paine D. & Cerniglia C.E. 2000. Mol. Cell. Probes, 14: 1-5.