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Review Article

Traceability and Instigation of DNA Marker Technology as a Tested Tool for Meat Traceability

Sanjay Kumar Bharti[1], V. Pathak[2], Anita[3], M. Goswami[4], T. Singh[5]

Author's Affiliation
[1,4]Assistant Professor [2]Professor and [5]Head PhD Scholar, Dept. of Livestock Products Technology College of Veterinary Science & Animal Husbandry U.P. Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Viswavidyalaya Evam Go-Anusandhan Sansthan, DUVASU, Mathura, Uttar Pradesh 281001, India. [3]Assistant Professor, Department of Livestock Products Technology, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttrakhand 263145, India.

Coressponding Author:
Sanjay Kumar Bharti, Assistant Professor Dept. of Livestock Products Technology College of Veterinary Science & Animal Husbandry U.P. Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Viswavidyalaya Evam Go-Anusandhan Sansthan, DUVASU, Mathura, Uttar Pradesh 281001, India. (U.P.) 281001- India.

E-mail: drskbharti@gmail.com

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Abstract

Globalization of market, consumer awareness and media attention on food safety issues such as Bovine Spongiform Encephalopathy and dioxin surge have eventually drawn attention of industry on meat traceability systems. Traceability is a contemporary concept able to ensure the quality and integrity by following a complex food supply chain route from raw material to the selling stage. Food regulations frame the principal of food safety and quality assurance, which radically prescribe traceability system in meat food business. Complexity of food production chains materialized meat animal rearers, processors, distributor and retailers for using advanced tools to ensure traceability. System of DNA analysis of meat and meat products is able to determine, unambiguously and with utter certainty, the origin of meat and a meat product. In other words, it can answer the question whether two biological samples (blood, meat, bone) originate from the same individual, from this individual's parents or descendants, or again from individuals. The DNA tracing technology on a meat product assures the products purchased are subjected to one of the most valid and accurate full traceability systems available, based on the current technologies in forensic science.

Keywords: Traceability; DNA markers; Meat, Regulations; Genetic traceability.

Introduction

In the past decades, distinct crises enforced the governments, regulators, businesses and consumers to acquire information respecting animal health, food quality and safety. Confront issues underlined the obligation to develop instruments that could assure reliable information throughout the food chain and could enhance food safety. Traceability in the food chain is presently achieving priority as it is an imperative factor in augmenting food safety policy (CEC, 2000). Steep incidences of contamination by E. coli, BSE, dioxin, hormones, and antibiotics have accorded a desire to find out groove for improving quality-control systems in the meat supply chain (Aung and Chang, 2014). Traceability in a modern concept acquiesce following a product's route from raw materials to the ultimate product for sale i.e. dodging full consideration on identifying, tracking and documentation procedures. This notion of traceability has gained compelling attention as it allows competent identification, correction or removal of risk factors throughout the food chain in order to bring only safe and quality products to consumers (Corina, 2013).

History of Traceability

The testimonies display that livestock farmers, owners, and those in charge of animal production and health were concerned with traceability from a very early stage. Although in lack of modern traceability techniques, our ancestors, as early as the 17th Century, exercised indelible branding and strict health certification. Animal identification by means of marking animals' bodies was recorded from way back. Animal products were likewise securely monitored, especially in the midst of human plague epidemic during 14th Century. Over the major epizootics of the 18th Century, some contaminated products (meat and hides) were cut up, slashed or covered with lime to pinpoint that the product is unsuitable for trade or utilization (Blancou, 2001).

Defining Traceability

By definition, traceability is as a system apt to maintain a credible custody of identification for animals or animal products through various steps within the food chain, from the farm to the fork (Ruiz-Garcia et al., 2010). As per, ISO 8402 standard norms, traceability is defined as ''the capacity of establishing a product's origin process history, use and provenance by reference to written records'' (ISO, 1994). The Codex Alimentarius Commission defines traceability as "the ability to follow the movement of a food through specified stage(s) of production, processing and distribution".

Traceability craves system for animal identification and registration and for labelling animal products, with the intent to ensure seam between the animal and the meat produced (Hong et al., 2011 and Caporale et al., 2001). Inflation of global trade, computerization and communications, plain language characterization of products and services, need to be recouped by identification and product tracing structure that are accessible in all trade and industry sectors worldwide. Now, animal producers and food suppliers already have at least some extent for tracing products. Various farmers and ranchers keep track of individual animals and how they are being grown. Traceability can help them to identify and exploit desirable production characteristics, such as animals that can grow more rapidly on limited feed or that yield a better cut of meat. Traceability supports to manage inventories, correspondent shipments and monitor consumer behaviour. Traceability enables the supply of food as per the preferences of consumer from the animals raised according to specified organic, humane treatment, or environmental standards. Traceability can avail firms to separate, and keep records on, these unique products to verify production methods.

Global Regulations and Voluntary Schemes

A Codex document (CAC/GL 60-2006) (Codex Alimentarius, 2006) elaborates a set of principles to assist competent authorities in exploiting traceability/product tracing as a mechanism within their food inspection and certification system this system can applied when and where appropriate in order to contribute to the protection of consumer against food free hazards (falls under WTO SPS agreement), deceptive marketing practices and facilitation of trade (falls under WTO TBT agreement). This document should be read in conjunction with all relevant Codex texts as well as those adopted by the International Plant Protection Convention (IPPC) and the World Organization for Animal Health (OIE), where appropriate (Codex Alimentarius, 2006). ISO 22005:2007 provides principle and standard for traceability implementing in the feed and food chain traceability system. It is intended to be flexible enough to allow feed and food organization to achieve identified objectives (ISO, 2007).

The Global Traceability Standard (GTS) is notified by GS1, an international not-for-profit association with member organizations in over 100 countries. GTS frames traceability systems available on a global scale, all along the supply chain, without bothering how many companies are involved or how many borders are crossed and what technologies are used (Porter et al., 2011).

GS1 standards provide the necessary framework need to support a seamless traceability system. GS1 Global Traceability Standard (GTS) was developed in 2005, with rolling participation of global industry which defines the globally-accepted method for uniquely identifying and sharing information on - trading items, logistics units, trading locations, trading partners, inbound and outbound shipments. GS1 Traceability standards enable compliance with all major global regulations such as ISO standards on traceability and recall, GAP (Good Agricultural Practice), EU Food Law, U.S. Bioterrorism Act and ACCP, among others (GS1 India, 2015).

Indian National Regulations

Public recalls of food products due to food safety concerns are comparatively rare in India, but with the thriving competence of the FSSAI (Food Safety

and Standards Authority of India) and with the development of branded food products they are becoming accepted tools to protect customers when things go wrong with a food production process. India is rather progressive in the food traceability area and has implemented various tracking and tracing systems in its food industry.

Introduction of Traceability Systems in India by APEDA in the form of Tracenet System, for enhancing the credibility of certification system of organic products, a user-friendly web- based traceability system has been implemented by APEDA since June 2010. This is world's first ever web based traceability system implemented at national level for organic products in contour with the National Programme for Organic Production (NPOP) where APEDA is the Secretariat and the accrediting body for accreditation of Certification Bodies (Tate, 2001). This Tracenet system helps in maintaining accurate information and analogous data of all the organic stakeholders under certification i.e. operators (producers, processors traders, ICS) and certification bodies operating under the NPOP (Thakur, 2015). The Tracenet system covers certification of all horticulture and agriculture crops including cotton, processed foods and wild harvest. Eventually, it will be further upgraded with the inclusion of Livestock products (meat, poultry, dairy, honey) and aquaculture products in the near future.

Meat.Net which was created by, The Directorate General of Foreign Trade (DGFT), Ministry of Commerce and Industry, Govt. of India vide notification No. 12/ (2004-2009) dated 21/12/2004 and DGFT notification no-82(RE-2010)/2009-2014, dated: October 31, 2011, has made it mandatory to have integrated abattoirs cum meat processing plants/ meat processing plants/ abattoirs registered with APEDA prior to export. In prospect of the procedure detailed in, Document No.: APEDA/ MPD/Registration/2013 dated: 6th December, 2013, for grant of registration certificate shall be adopted. Despite enforcement mentioned procedures in force, there have been few cases where it has been highlighted the export undertaken by some of the exporters were more than what has been sourced by them from the authorized meat plants.

Methods of tracing

In order to ensure the safety, distinct kinds of traceability system have been built in many countries, in form of ear-tag (Stanford et al., 2001 and Caja et al., 2006), barcode labeling, branding, tattooing (Schembri et al., 2007), stamping, radio frequency identification (RFID) (Wang, 2014), retinal scanning combined with trolley-tracking (Smith et al., 2005b and Crandall et al., 2013) and DNA traceability (Nicoloso et al., 2013 and Galimberti et al., 2013). Subcutaneous transponders (Silveira, 2013) raise questions of welfare and the risk entering the food chain. Prola et al. (2010) studied retention rate and functionality of electronic identification systems in pigs, injected in different sites, evaluate traceability of animals and found injectable transponders in the intraperitoneal position provided the best identification system for pigs. Exquisite ideas such as injecting a unique antigen into the pigs from each farm to give readable antibody in the meat may find even less favor with the consumer (Webb, 2004). Inside the slaughter and processing plant, the simpler choice include paper bar codes (Pedro et al., 2011) that can be read and reprinted at each point where a cut is divided into smaller portions (Webb, 2003). Batch can be identified by some form of "marker" or interruption that passes through all lines within the plant. More upscale options include RFID or smart credit card type systems (Piramuthu and Zhou, 2016 and Yordanov and Angelova, 2006). DNA traceability system is relatively advanced and reliable for the traceability of retail meat among these technologies (Galimberti et al., 2013).

The cost of identification and thereby traceability setup vary extremely, depending on the method applied and the level of detail required. Operational traceability schemes are often a precondition for meat producing countries to enter export markets. Hence traceability is not only a health issue but has also become a marketing tool (Marucheck et al., 2011). The ultimate aim is to achieve transparency in the meat chain "from farm to fork" (Wognum et al., 2011).

Genetic Traceability

As the name submits, genetic traceability is based on the study of DNA for the identification of animals and their products. In fact, DNA molecule has the characteristic of enormously variable between individuals (expect for monozygotic twins and clones) allowing to distinguish betwixt (Mackie et al., 1999 and Cunningham and Meghen, 2001). Apart from it DNA also have some unique features like:

- DNA is inalterable throughout animal life (Barcaccia et al. 2016)
- DNA is stable during contrast treatments of food processing (Chiter et al. 2000)
- DNA is present in every cell of the organism (David et al., 2000)

DNA can be procured from the preferred matrix (it can either be animal tissue, blood, muscle, hair, sperm, faces (Stray et al., 2010) or even a processed food such as cheese or canned meat) and is analyzed by the use of molecular markers to draw a fingerprint (Vignal et al., 2002) or specific allelic frequencies allowing approach for individual, breed or species identification. Considering the introduction of the polymerase chain reaction (PCR) in 1989, several distinct markers have been discovered and investigated. A genetic marker is a gene or DNA sequence with a known location on a chromosome that can be used to identify cell, species or individual. Nowadays the most widely used markers are microsatellites also termed as short tandem repeats (STR) and single nucleotide polymorphism (SNP) that contain short sequence of DNA (Mariani et al., 2005). The use of these technologies in animals and their products is just a purview of techniques already ongoing for human testing and routinely practiced in forensic investigation (Cunningham and Meghen, 2001).

Genetic Markers

Multi-locus Markers

1. Minisatellite or VNTR (Variable Number Tandem Repeat) markers:

Minisatellites, discovered by Jeffreys et al. (1985) in human DNA structure (Avise et al., 1989), were the first markers with sufficiently informative that each individual had a unique genotype. These are chiefly dominant markers with only one allele able to be identified. A few, highly informative, single loci minisatellites identified in livestock (Georges et al., 1990) have been found useful. However, with the advent of microsatellites they have fallen out of favor because they tend to be biased in their distribution within the genome and are often clustered near telomeric region of chromosome (Royle, 1988 and Wells et al., 1989).

2. RAPD (Random Amplified Polymorphic DNA fragments) markers:

RAPD markers were proposed as a PCR based molecular system (Willams et al., 1990). These are very simple to generate and require only small quantities of DNA. These are the fragments generated in PCR reaction using a short single primer of arbitrary nucleotide sequence. Amplified products are analyzed by gel electrophoresis and polymorphism is detected by presence or absence of DNA fragment. This system sometime seems to be imprecise as appearance and disappearance of DNA is very sensitive to slight changes in PCR conditions (Dodgson et al., 1997).

3. AFLP (Amplified Fragment Length Polymorphisms) markers:

AFLPs are now the multi-locus markers of choice and belong to the category of selective restriction fragment amplification (Vos et al., 1995), which is based on the ligation of adopters, to genomic restriction fragments followed by PCR based amplification with adapter specific primers. AFLP achieves a high multiplex ratio and does not require prior information of the DNA sequence of the genome under study (Meudt and Clarke, 2007).

Single Locus Markers

1. Restriction Fragment Length Polymorphisms:

RFLPs were the first DNA markers developed and used to visualize differences in the structure of DNA. The RFLP methodology utilizes restriction enzyme digestion of the genomic DNA (Grodzicker et al., 1974), its separation by size using agarose gel electrophoresis and detection and analysis of the DNA sequence by a technique called Southern blotting (Sambrook et al., 1989). RFLP markers disclose the presence or absence of a restriction site, including insertions and deletions of additional sequences, provided it have an additional restriction site or change the band size seen on the Southern blot.

2. Microsatellites:

Microsatellites are multi-allelic tandem repeats (Oliveira et al., 2006). However, they are single locus, co-dominant, spread throughout the genome, requiring only small amounts of template DNA and are relatively easy to find and characterize. They are usually considered as evolutionary neutral DNA markers and interspersed throughout the genomes of every organism analyzed so far (Li et al., 2002). A microsatellite is a simple sequence that is repeated 10–50 times. Virtually all of the microsatellites found in livestock have the sequence (AC/GT) as the repeat unit as it is the most abundant type within livestock genomes and therefore much easier to find and characterize.

3. Single Nucleotide Polymorphisms:

The variation in sequence at a particular position is called a single nucleotide polymorphism (SNP). They refer to genetic variation at the lowest possible level; the single base or nucleotide (Cooper et al., 1985). SNPs represent by far the affluent source of genetic variation available for traceability applications. However, SNPs are almost always bi-allelic (Vignal et al., 2002) and much less informative individually than the multi-allelic markers and differentiation between individuals requires many more SNPs to be analyzed than highly informative multiallelic markers. The development of new technologies, such as "DNA chips" accommodating high density arrays of DNA (Chee et al., 1996), along with establishment of SNP maps and libraries allows many SNPs to be precisely genotyped at once.

DNA tracing works in practice

DNA tracing system starts at farm where animals enter for commercial production. A blood sample is DNA-typed for the genetic marker panel and the identity information is entered in a database. Producers commonly receive bar-coded blood tubes, together with a CD-ROM containing details of Internet access. The breeding female's identity is maintained on a sheet beside the barcode, and is posted along with the tubes to the DNA testing laboratory. The laboratory types the sample and enters the dam's DNA genotype and farm into the database. The producer updates the database directly with farrowing and culling dates. Meat samples are sent to the laboratory, and the DNA genotype is entered into the database. Meat is then matched to the mother's identity for animal farm, date of birth, sex, feeding or any other relevant information extended on the database by a computer search engine. A batch live animal tracking system is then link with the support of an identification device that provides online and web feedable information on individual animal basis.

Advantages and limitations of DNA tracing

DNA systems have some key supremacy for traceability:

- DNA, fundamental part of the meat and meat products. DNA character cannot be destroyed without destroying the product (Tate, 2001).
- DNA gives extreme towering standard of testament from an arbitrator (DNA testing laboratory) that is difficult to match with intra-company paper or data records (Visayadamrong et al., 2013).
- DNA profiles are inherited genetically and enclose other information, i.e. parentage, breed, species, presence or absence of specific gene variants.
- DNA traceability is simple to implement because it only involves taking and storing a sample. The system does not obstruct with workflow or require restructuring of processing procedures (Daria and Rosa, 2014).

One of the main limitations of current DNA systems is that results are not immediately available (Arana et al., 2002); the process of locating and shipping samples and then conducting DNA analysis can take days. This perhaps is a detriment if rapid response is required in a product recall situation. A second limitation is the expense of DNA profiling. Although sample archiving is routine, current systems only trace animals on an audit or "as required" basis. DNA systems will only work well with accurate batch recording so that, the DNA search is limited to relatively few samples and if no DNA match is detected, a firm can categorically state the product is not from their system. To some extent a good batch recording system provides course of immediacy in DNA tracing. Immediate recall decisions can be made on batch information, with individualisation from DNA refining this process at a later date (Tate, 2001).

Conclusions

Traceability of livestock products is a crucial means to safeguard public and animal health, and to valorise typical foods. Due to its high accuracy and use of genetic information, DNA can be used to audit other tracking Systems. At present DNA based techniques seem to be the applicable tool for the verification of the origin of animal products and research has made tremendous improvements in the last few years, moreover, these techniques are already used for human testing in forensic cases. Traceability is increasingly recognized as key risk mitigation and management tool, as well as a critical component of quality assurance in the agri-food industry. Within the livestock sector, animal identification - a key requirement for traceability is be-coming mandatory in many regions of the world. All farmers are food producers just the same

as processors; wholesalers, caterers and retailers, and they play a vital role in the production of food which meets the apical standards of hygiene and safety. The chain is only as strong as its weakest link and farmers, as the first link in this chain, need to be aware of all relevant food safety issues and need to able to exhibit that they operate safe production systems. For the system to operate effectively an auditable production trail is necessary and the processor must pursue standard operating procedures to avoid contamination. Providing these conditions are met, the standard analytical methods will match a DNA sample, taken from a meat-cut in the marketplace, to the DNA profile of a carcass at slaughter. Finally, social and economic drivers and human behaviour remain a significant challenge. Any traceability system, no matter how carefully constructed can be circumvented. In this context, it is important that traceability systems are not simply imposed as a cost but are built to provide commercial value and feedback for producers and processors willing to modify practices, or breed animals to valued market requirements.

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