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Meat Species Specifications to Ensure the Quality of Meat-A Review

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ABSTRACT

Meat species specification is an area which needs specialized attention in the food quality management system. It is a vital field to ensure the food safety to the consumers and it conserves the laws related to meat and meat products. The adulteration of inferior quality meat into superior quality meat is a common practice all over the world. Using meat species specification techniques we can easily solve the problems of vetro-legal or forensic cases particularly related to Prevention of Cow Slaughter Act, Prevention of Food Adulteration Act in India and related laws existing worldwide. Various methods are available right from physical, chemical, anatomical, histological and biological to sophisticated molecular techniques. All these methods have pros and cons so the knowledge of all these techniques is of immense value for the persons having some stakes in this specialized field. If the meat is in carcass form, it can be easily identified with the physical, chemical, anatomical and histological methods but the reproducibility and quantitative identification is not possible. In biological methods we use the simple method of antigen-antibody reaction for visual identification. In electrophoresis, the migration of the protein moiety according to their molecular weight under the influence of electric field principle is applied. The band patterns produced in this technique is them visualized for result interpretation. In recent molecular techniques DNA and RNA amplification is done to produce the fingerprints as per the characteristics of an identical genetic material for a particular meat species. Now the development of PCR technique makes easy to identify the meat species even from the cooked and spoiled meat in which protein is easily destroyed. Real time PCR is the revolution in this field in which we can identify and monitor the product during its amplification. Although no single technique is sufficient for differentiation of all types of meat species and meat products.

Key words: Meat, meat species specifications, finger printing, amplification, adulteration, PCR

INTRODUCTION

Meat species specification is an utmost important field of quality control management in meat industry. It is more challenging and revolutionary task for us as a veterinarian because by this way we can ensure the quality of meat and also helps in conservation of law existing in different countries. These tests are very much important for accurate detection of meat species and their fraudulent substitution in another meat (Singh and Sachan, 2009). These practices are also helpful in implementation of prevention of cow slaughter acts of different states of India, wild life conservation act, PFA acts of India and some other similar acts of the world (Singh and Sachan, 2010).

Fraudulent substitution is a malpractice in meat industry, in which inferior or cheaper quality meat is mixed into superior quality meat. These practices are not new but existed since long back as the first case of fraudulent substitution was recorded in thirteenth century A.D. at Florence in Italy (Thornton, 1968). A common fraud in the meat industry in which uninspected meat is substituted for meat that has undergone inspection and been branded as satisfactory. The other frauds are the substitution of meat of another species, i.e., horse for beef especially in Britain and Ireland, beef in kangaroo meat in Australia, cat for chicken or rabbit, goat meat for mutton, mutton for venison, dog meat and cat meat for chevon (Kang'ehte *et al.*, 1986) in other countries including India. As per an estimate about 25-30% of meat sold in India is adulterated. These practices are more common in comminuted meat products. For detection of meat species in adulterated meat have several techniques started from simple physical tests to recent sophisticated molecular techniques.

Physical techniques: In physical techniques we generally go for general appearance for detection of different meat species. It is a combined perception of colour, texture, odour and presence of other body parts along with meat. It gives the primary idea about the meat species on the basis of quality characteristics of the meat. The detailed information regarding physical characteristics of meat and fat (Gracey *et al.*, 1999; Sharma, 1999; Singh, 2008a, b, 2010; Singh and Sachan, 2010; Sachan and Singh, 2010) is presented in Table 1 and 2 simultaneously.

As far as prevention of cow slaughter act is concerned we will have to identify the meat of beef with other closely related species particularly with buffalo meat that is most commonly encountered case in India (Singh, 2010). The differentiation of beef and buffalo meat can easily be done on the basis of physico-chemical characteristics as shown in Table 3.

Anatomical techniques: On the basis of anatomical structure of different animal's species using for meat production, we can easily identify the meat species to which it is actually belongs (Gracey *et al.*, 1999; Sharma, 1999; Singh, 2008a, b; Sachan and Singh, 2010). But these anatomical structures must be present on the meat at the time of meat inspection. It is not a method for meat species specification if meat is in lean. The primary identification method for meat species is dental formula if teeth are attached with the carcass. The typical dental formulations are given in Table 4. Another anatomical technique for carcass identification is on the basis of vertebrae and

Table 1: Quality characteristics of meat of different animals (Singh, 2008a, b, 2010; Sharma, 1999)

Meat	Colour	Consistency	Odour	Marbling
Beef	Dark red with slight brownish tinge	Firm and cut surfaces are shiny	-	Present
Buffalo meat	Dark red	Firm	-	Absent or poorly present
Veal	Pale grey to grayish red	Firm	-	Absent
Chevon	Light red and paler than mutton	Very firm	Goaty odour	Absent
Mutton	Dark red	Firm and dense	Ammonical	Absent to scanty
Pork	Grayish white to light red	Very soft	Urine like	Present
Poultry meat	White	Firm	-	Absent
Horse meat	Dark red with bluish tinge	Firm with prominent fascia	-	Absent
Camel meat	Red	Fairly firm	-	Absent
Dog meat	Dark red	Firm	Disagreeable and repulsive	Slightly present
Rabbit meat	Pale, grey to grey red	Firm	Pronounced	Absent
Venison	Dark red to brownish red	-	-	Absent or very less

Table 2: Quality characteristics of fat of different animal species (Singh, 2008b, 2010; Sharma, 1999)

Fat	Colour	Consistency	Fat type	Bone marrow characteristics	Remark
Beef	Yellowish white	Firm	Intramuscular fat	Pure white to reddish yellow	-
Buffalo fat	Pure white	Slightly firm	No Intramuscular fat	-	-
Veal	Reddish yellow to white	Loose and greasy	No Intramuscular fat	Pink red	-
Chevon	Pure white	Hard , firm and brittle	No intermuscular fat	Firm and slightly red	-
Mutton	Pure white	Hard , firm and brittle	Abundant intermuscular fat	Firm and slightly red	-
Pork	White	Soft and greasy	Subcutaneous but	Pink red and soft intramuscular also	On boiling it turns to whitish grey
Poultry fat	Yellow	Loose	Mostly subcutaneous	-	-
Horse fat	In young-light gold to yellow In mature -white	Soft and greasy	No intramuscular fat	Waxy, yellow, greasy and soft	On exposure to air turns to blackish
Dog fat	White to whitish grey	Oily and greasy	Slight intramuscular	-	-
Rabbit fat	Whitish yellow	Loose	Fat is absent in muscle and confined to body cavity	-	-

Table 3: Differentiations of beef and buffalo meat on the basis of physical and chemical characteristics (Singh, 2008b; Singh and Sachan, 2010)

Characteristics	Beef	Buffalo meat
Colour	Dark red with slight brownish tinge	Dark red
Meat consistency	Firm and cut surfaces are shiny	Firm
Marbling	Present	Absent or poorly present
Fat colour	Yellowish white	Pure white
Fat consistency	Firm	Slightly firm
Location of fat	Intramuscular	Mostly intermuscular
Carotene content	Present	Absent

Table 4: Dental formula identification of different meat animal species

Animals species	Dental formula	No. of teeth
Cattle and buffalo	(0033) 2 -------- (4033)	32
Sheep and goat	(0033) 2 -------- (4033)	32
Pig	(3143) 2 -------- (3143)	44
Horse	(3133) 2 -------- (3133)	40

ribs number present on the carcass. The specific numbering of vertebrae and ribs found in different meat species are shown in Table 5.

In some countries of the world slaughtering of cows and horses for meat production are common practices. So, their adulteration may create the problem of quality assurance. In those cases

Table 5: Specific characteristics of vertebrae of different meat animal species and ribs (Singh, 2008a, b; Singh and Sachan, 2010)

Type of vertebrae	Cattle and buffalo	Sheep and goat	Horse	Pigs	Chicken	Rabbit
Cervical	7	7	7	7	15-17	7
Thoracic	13	13	18	14-15	7	12
Lumber	6	6	6	6-7	L+S fused14	7-8
Sacral	5	4	5	4		3-4
Coccygeal	18-20	16-18	15-21	20-30	5-6	14-20

Table 6: Differentiations of horse meat and beef on the basis of anatomical structures (Singh, 2008a, b; Singh and Sachan, 2009, 2010)

Characteristics	Horse meat	Beef
Vertebrae characteristics		
a. Superior spinous processes of first six dorsal vertebrae	Well developed	Not well developed
b. Transverse processes of last three lumber vertebrae	Articulate with each other	No articulation
Ribs	Narrower but more markedly curved	Comparatively thicker and less curved
Thoracic cavity	Longer due to 18 ribs	Comparatively smaller due to13 ribs
Specific characteristics of long bones		
a. In fore quarter		
Ulna	Extends half of the length of radius	Extends full length of radius and articulates with carpus
b. In hind quarter		
i. Femur	Third trocanter is present	Third trocanter is absent
ii. Fibula	Extends 2/3rd length of tibia	Fibula is a small pointed projection

Table 7: Differentiations of Mutton and Chevon on the basis of anatomical structures (Singh, 2008a, b; Singh and Sachan, 2009, 2010)

Characteristics	Mutton	Chevon
Vertebrae characteristics (Lateral border of sacrum)	Thickened in form of roll	Thin and sharp
Thorax	Barrel shaped	Laterally flattened
Specific characteristics of long bones		
a. In fore quarter		
i. Scapula	Short and broad, superiors spine, bent back and thickened	Have distinct neck, spine straight and narrow
ii. Radius	1.25 length of metacarpus	Twice is the length of metacarpus

anatomical differences among theses species may be the important tool. Some important anatomical variations among these animal species are presented in Table 6. Mutton and chevon are the other meats which are frequently encountered in meat quality assurance system. They are closely related animal species and may be confused easily. So, some anatomical differences among these animal species give an idea about their presence in another meat type. The major anatomical structural differences among these closely related animal species may be cited from Table 7.

Sometimes to conserve the interest of consumers we will have to identify the meat of male and female animals of different species. For that purpose we can identify the meat of male and female animals if the meat sample is presented in form of carcass or cuts. It may be differentiated by presence of gonads, udder and developed gracilis in females and bulbocavernosus muscle in male. The size of pelvic cavity in female animal's carcasses will be larger than male animals.

Histological techniques: In this technique we generally measured muscle fiberlength, diameter, density and pattern of the muscle fibers in different meats of animal origin. Frequently

Table 8: Differentiations of Beef and Buffalo meat on the basis of histological parameters (Lawrie and Ledward, 2006; Singh, 2008b)

Characteristics	Beef	Buffalo meat
Muscle fiber diameter	Larger	Smaller
Number of muscle fibers per mm^2	Less	More
Muscle striation	Less angular	More angular

Table 9: Chemical characteristics of meat of different animal species (Lawrie and Ledward, 2006; Singh, 2008b)

Tests	Buffalo meat	Beef	Mutton and chevon	Pork	Horse meat
Values in meat					
Glycogen	0.5 to 1.0%	0.5 to 1.0	0.5 to 1.0	0.5 to 1.0	2.28%
Intramuscular fat	0.9%	2.6%	In mutton 13.3% while in chevon 3.6%	4.4%	Nil
Values of fat					
Linoleic acid	0.1%	0.1%	0.1%	0.1%	1-2%
Carotene test	Nil	0.14-0.225 mg g^{-1} of fat	-	-	-
Refractive index	-	40	41.5	51.9	53.5
Iodine value	-	38-46	35-45	50-70	71-86

encountered case of cow and buffalo meat mixing and illegal slaughtering may be identified by the histological techniques. Some basic differences in these species meats are compared in Table 8.

Chemical techniques: For meat species specifications various chemical tests are of immense value. In these tests we can easily estimate the amount of certain chemicals presents in the meat of different animal species (Thornton, 1968). On the basis of its contents present in particular meat we can easily get an idea about the meat species. Carotene contents present in beef and buffalo meat may fulfill the need meat species specifications in case of prevention of cow slaughter act in India. The values of different compounds typically found in different meat animal species are given in Table 9.

Biological techniques: These techniques are mainly based on the principles of antigen antibody reactions. The homologous antigen binds with the antibody which is visualized by various methods. These techniques are simple and can be performed any where with little efforts. These tests are also known as Serological or Immunological methods.

Precipitation test: It is also known as Ring Precipitation Test (RPT) in which homologous antigen and antibodies reacts together and make a ring at the point of its interaction. Known antibodies (blood serum) collected from the treated experimental animals are mixed in a test tube with the filtered extract of meat samples. If precipitation line forms at the point of mixing then it indicates positive test. For example if the antiserum of horse mixed with filtered extract of suspected meat in a test tube, a turbidity occurs and forms a definite precipitation ring if sample is positive for horse flesh. It has some limitations such as, this test can applied only for qualitative evaluation; can not apply for heat treated meat. It gives false positive test in closely related species due to cross reactivity. In this technique developed ring is difficult to observe and diffused in a short period of time.

Complement fixation test (CFT): CFT is also known as Immunodiffusion Test or Agar Gel Precipitation Test (AGPT) or Agar Gel Immunodiffusion Test (AGID). The principle of test is same as in precipitation test but a compliment is used to reproduce the results for longer duration. For

this purpose Guinea pig serum as a complement source and the sheep RBCs and rabbit serum as an indicator are used. This test can be used both for qualitative and quantitative test, adulteration up to 5% can be detected as well as by this method meat cooked at 80°C for less than 10 min can be detected. It also has some additional benefits like detection of 1:32 dilution of the sample may be possible. The slides prepared in this method can be preserved for future use. However, it is a time taking method (about 2-3 days) and ineffective in case of thoroughly cooked meat. It also has low sensitivity and may give false positive results in closely related species due to cross reactions.

Overnight rapid identification tests: These tests are Overnight Rapid Beef Identification Test (ORBIT) or Poultry Rapid Overnight Field Identification Test (PROFIT) or Multispecies identification field test (MULTI-SIFT) or Dot-blot technique. In these tests three disks, i.e., blank, disk with beef or poultry antigen and disk with bovine or poultry antiserum are generally used. The sample fluid is filled in blank disk and directly placed in precast agar gel and allow it to incubate overnight and then observed for precipitation development. This test can also be used for multispecies by using Multispecies identification field test (MULTI-SIFT). This modified test can be used for beef, pork, poultry, sheep, horse and deer meat. Another similar test is Dot-blot technique in which binding of antigen from the sample is take place in a membrane (nitrocellulose or cyanogen bromide activated nitrocellulose) with a specific antibody. It is a rapid and sensitive test but it is not sufficient method for quantitation (Jones and Patterson, 1985).

Enzyme-linked immunosorbent assay (ELISA): It is a rapid, highly sensitive(able to detect 2% adulteration) and most suitable method for handling numerous samples at a time. By this technique we can detect up to 2% adulteration in the sample (Govindarajulu, 1989). There are several ELISA techniques are in use depending on the compound fixed, solid support used and concentrations of antigen and antibodies used such as Indirect ELISA, Competitive ELISA and Sandwich ELISA (Patterson and Spencer, 1985). In Indirect ELISA, species is detected by the antisera that are subsequently used and labeled with suitable conjugate. While, in Competitive ELISA a fixed amount of antigen antibodies are mixed with the meat extract and preincubated. These techniques are mainly based on the polyclonal antibodies against muscle or serum protein. These polyclonal antibodies are limited in production, have heterogeneous affinity and needs purification to avoid cross reactions. So, the use of monoclonal antibodies is most common in ELISA test because they are specific for a single antigenic site. These monoclonal antibodies can be produced from thermostable proteins of different species or by the hybridoma cell lines. It is a rapid test and results may be obtained within 2-3 h. By this technique we can detect closely related species with the capability of testing numerous samples at a time. This test is also able to detect pressure cooked meat at 133°C for 20 min.

Electrophoresis techniques: These techniques are based on the separation of proteins by their differential migration through a supporting medium under the influence of electric field (Kim and Shelef, 1986). The protein bands thus resolved are visualized by general, enzymological, chemical or immunological means. For this test sample is prepared by homogenization with 10 volume of 0.03 N phosphate buffer (pH 7.4). Then sarcoplasmic proteins are extracted with 0.1 N phosphate buffer and myofibrillar protein with 1.1 M KI (pH 7.4) by centrifugation at 10000 rpm for 20 min. The supernatant is then filtered which can be used with gel of electrophoresis.

Poly acrylamide gel electrophoresis (PAGE): Initially disc electrophoresis in polyacrylamide gel was used for meat protein separation on the basis of total protein pattern of different meats. A simple visual differentiation without staining of the species is generally practiced to compare the respective migration rates of the brownish myoglobin bands. But it is a time consuming process and bands diffused early. So, the PAGE was shifted to slab gel method. Myoglobin and creatine kinase Isozymes bands obtained in thin layer agarose gels have also been used for detection of beef, pork, chicken and turkey.

But now a days PAGE is carried out in cello gel strips. The process involves three main steps, i.e., extraction, electrophoretic migration and staining-destaining. The stain commonly used in this method is amido black. In this process several blue bands forms which may differ in its width and intensity of staining. In a particular species distribution of band and their intensity of staining are stable and characteristic and can be examined by naked eyes. On the reading of destained strips through photometer with integrator or pherogram showing different peaks in which width of the bands on the gel and length proportional to the intensity of staining are species specific. This method is useful for fresh as well as for frozen meat identification.

Sodium Dodecyl Sulphate PAGE (SDS-PAGE): SDS-PAGE is a variant of PAGE is commonly used for separating protein subunits and determining their molecular weights. On heating, polypeptides dissociate and when these polypeptides bind with SDS in the presence of reducing agent 2-mercaptoethanol forms SDS-polypeptides. When this complex is subjected to a sieving polyacrylamide gel, migrate according to the molecular weights of the polypeptides. PAGE-SDS (pH 3-10) can be utilized for identification of beef, mutton, venison, rabbit meat and raw and cooked crustaceans. It is a suitable method for heated meat samples up to 100°C beyond which most of the protein bands disappears. This is a good technique for closely related meat species and has good resolution and reproducibility.

Counter immunoelectrophoresis (CIE): CIE is an immunodiffusion test in which electric voltage is used for acceleration of protein movement. In this technique alkaline gel is used which causes electro-osmosis. Under this type of electrophoresis antibodies and proteins from meat extract, moves towards each other and in case of homologous condition they forms a precipitation band at the point of its joining. By this technique we can detect one part of species in 300 of meat; it is rapid and more sensitive test (Sherikar *et al.*, 1988).

Isoelectric focusing (IEF): IEF is an electrophoretic technique in which charge is utilized at surface of protein to drive it through gradient gel. It is mainly based on the migration of proteins in a pH gradient. The process stops when the surface charges become neutral, at isoelectric point. The subsequent fixing makes the protein precipitated and fixed at the same point as bands. The formation of such bands is species specific and can be utilized for meat species identification by determining the location, density and area of the bands. It is a suitable method for identification of animal species even after cooking at 100°C but not suitable for closely related meat species (Skarpeid *et al.*, 1998). It is also not good for frozen meat condition and results are difficult in interpretation and have poor reproducibility.

For better visualization of band patterns in case of lower proportion of contaminants certain enzyme stain can be used (King, 1984). The examples are coomassie blue for whole muscle samples, phosphoglucomutase for low levels of buffalo, pig or horse meat in beef, adenylate kinase for low

levels of kangaroo or horse meat in beef, Phosphor Gluconate Dehydrogenase (PGD) for differentiation of mutton from chevon.

Immobiline gels and immunoblotting: In this technique electro-focusing is done in immobiline gels contrary to carrier ampholytes in tradition electrofocusing methods. The immobilines are derivatives of acrylamide which produces immobiline pH gradients with the co-polymerization of acrylamide. The covalently bound immobiline gradient allows higher resolution than conventional carrier ampholyte based pH ingredients. The electrophoretic transfer of focused proteins from polyacrylamide gel to nitrocellulose membrane in order to make them accessible for identifying reaction is known as immunoblotting.

Molecular techniques

Cation exchange chromatography: A simple sample preparation procedure consisting of an extraction step with Milli-Q water as extraction solvent for hemoglobin's from meat samples is followed. The filtration is done with a cellulose acetate filter. Then cation exchange chromatographic separation is done and diode array detection obtained. Then different peak patterns for extracted hemoglobin's of different species may be obtained. Other heme-group containing proteins like myoglobin or cytochrome C which may also be detected with diode array detection at 416 nm. These may be chromatographically separated from the hemoglobins. By the use of these characteristic peak patterns, the species of the meat can be specified (Ashoor *et al.*, 1998).

DNA based molecular techniques: DNA is molecule of choice for species specifications due to its stability during heating and processing. DNA molecular based species specification is possible in the foods obtained from identification rendered meat products, genetically modified foods etc.

DNA hybridization technique: It is a qualitative or semi-quantitative technique of meat species speciation in which species-specific DNA sequence is detected (Ebbehoj and Thomsen, 1991). For this purpose probes prepared from DNA or cloned DNA are hybridized with target DNA and detected by colour development or audiography. During the early development of DNA sequence analysis, genomic DNA was used as a species specific probe and was hybridized to DNA extracted from meat samples. The subsequent development of probes derived from species-specific satellite repetitive DNA sequences has greatly improved the specificity of the assay, now making it possible to detect admixtures that contribute as little as 5% or less to a product (Wintero *et al.*, 1990). An alternative DNA detection system is based on the Polymerase Chain Reaction (PCR) amplification of a segment of the mitochondrial cytochrome b gene. Subsequent cleavage by a restriction enzyme gives rise to a species-specific pattern on an agarose gel. This method does not require the development of species-specific probes and, because it is PCR-based, is most suitable for critical samples in which DNA is largely degraded (Chikuni *et al.*, 1990). This is a good technique for detection of adulteration as low as 0.1% (Janssen *et al.*, 1998).

Polymerase Chain Reaction (PCR): PCR is a rapid method because in this technique we can obtain multiple copies of specific piece of DNA sequence *in vitro* and it has high degree of selectivity and sensitivity. PCR amplifies a target DNA sequence in an exponential phase, being capable of detecting even a single copy sequence from a single cell sample. It is a qualitative test for meat

species specification. There are two main techniques for amplification of genetic marker, i.e., mono-locus-specific primers for amplification of a concrete DNA fragment and multi-locus amplification of non-targeted DNA. By this technique closely related meat species can be identified with the discrimination between male and female raw meat.

PCR techniques using multi-locus primers: In this technique DNA amplification is used to produce fingerprints. Most commonly used techniques are Random Amplified Polymorphic DNA fingerprinting (RAPD) and Amplified Fragment Length Polymorphism.

Random amplified polymorphic DNA fingerprinting (RAPD): It is a modified PCR technique in which DNA fingerprints are generated in a very short period of time which can be visualized on gel electrophoresis (Lee and Chang, 1994). In this method prior knowledge of DNA sequencing is not required but known standard has to run each time. In this technique DNA Extraction from sample is the first step. Then it is amplified with specific primers. It is followed by denaturation of strand at 94°C for 1 min then primer annealing at 47°C for 1 min. There after extension of primer is take place at 72°C for 1 min. Electrophoresis (10 µL portion of the amplification) is carried out for 45 min at 100 V in a 3% agarose gel containing ethidium bromide (1 µg mL^{-1}) in TBE buffer (0.045 M Tris-borate and 0.001 M EDTA with pH 8.0). At last DNA fragments are visualized by UV transillumination (Calvo *et al*., 2001).

Amplified fragment length polymorphism: In this technique about 500 primer combinations produces selected markers which are converted into a single nucleotide marker for highly specific genotyping of the meat species (Meyer *et al*., 1995).

PCR techniques using mono-locus primers: These are mainly based on amplification of target DNA either by the use of species specific primers or use of universal primers. The prerequisite of this test is the prior knowledge of nucleotide sequence to be used as a target.

Species-specific PCR: In this technique species-specific DNA in femto grams (fg) and pico grams (pg) can be detected in both processed and unprocessed meat samples by using targeted amplification of rRNA genes (12, 16, 18 S), actin-multigene which is highly conserved in eukaryotes, satellite DNA, cytochrome-b gene, cytochrome oxidase-II, growth hormone gene, melanocortnin gene, mt D-loop, myofibrillar components and satellite I DNA using PCR. By this technique very old samples even of more than 100 million years can also be identified (Girish and Nagappa, 2009).

Multiplex PCR: In this technique many targets are simultaneously amplified which helps in detection of many species in a short period of time. By this technique organ meat can also be detected like mixing of duck liver in goose liver can easily identified. For that purpose multiplex PCR with common forward and species- specific reverse primers to nuclear repeated target (5S rDNA gene) is used.

Nested and hemi nested PCR: This technique can also be used in the meat showing spoilage. But in such case two stage PCR is generally used. In first stage low stringency primer pair is used for amplification while in second nested PCR technique is applied. By using polymorphism in mitochondrial 16S rRNA gene, meat of several wild animals can be detected at a time.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP): It is a modified form of PCR in which conserved target gene is amplified and resultant PCR product is digested with specific restriction endonuclease enzyme to get a restriction pattern or fingerprints. This is also suitable method for identification of even degraded DNA with apomorphic sites (Bauer *et al.*, 1987; Borgo *et al.*, 1996; Girish *et al.*, 2005). The first step in this technique is extraction of DNA then these DNA fragments are amplified with the help of DNA polymerase and specific primers. It is followed by sequencing of PCR products at restriction endonucleases sites then it is digested with restriction endonucleases. Last steps are separation by gel electrophoresis and detection of stained band. It is suitable method for the analysis of very low amount of meat (1 mg). It is a faster and more sensitive method than DNA hybridization. By this technique two species of animal can be differentiated even after heating at 120°C. In this technique proper identification of male and females can be done by using PCR and genomic DNA extraction from raw muscles (Lahiri *et al.*, 1992). It is not hundred percent accurate methods because of existence of some intraspecific variability with in the species as in sheep, turkey and chicken meat.

Forensically informative nucleotide sequencing (FINS): In this technique conserved region of gene specific to a species is amplified and the PCR product is cloned, sequenced and the sequence is analyzed. For this purpose 12S rRNA gene sequence analysis is the most preferred method for differentiation of beef, buffalo meat, mutton and chevon (Murray *et al.*, 1995; Girish and Nagappa, 2009).

Polymorphism techniques: These techniques are Direct Amplified Length Polymorphism (DALP), Single Strand Conformation Polymorphism (SSCP), Simple Sequence Repeat Polymorphism (SSRP) and Inter-Retrotransspoon Amplified Polymorphism (IRAP). In DALP, AP-PCR technique is applied to produce genomic fingerprints which enable sequencing of the DNA polymorphism for any species. While, SSCP is a secondary structure analysis tool in which single stranded molecules differing little similar to single base substitution and forms different conformers and migrate differently in non-denaturing gel electrophoresis. SSRP is a delicate technique which required complex instruments in using >40 primer sets. In the last technique retrotransposons are analyzed in sample genome which produces reproducible band patterns in their geographical origin on amplification with specific primers.

DNA invader assays: In this technique generic enzymatic method and Fluorescence Resonance Energy Transfer (FRET) methods are used for signal amplification.

Real time PCR: In this technique PCR products are detected and monitored during amplification in the same reaction vessel with the help of fluorescent compounds. This technique makes identification and detect of the product during its amplification (Ghatak and Gill, 2009). In this technique quantification of the meat species may be possible.

Although, several methods are available for meat species specifications but no single method can applied in all cases. As the physical, chemical and anatomical methods are more suitable for raw meat while minced or comminuted meat requires sophisticated techniques. For this purpose DID, SDS-PAGE, ELISA and IEF are common methods. Among these DID is not sufficient method for closely related meat species, effectiveness of ELISA and SDS-PAGE is hampered by cumbersome process of isolating species-specific proteins, IEF presuppose that the protein composition of meat

is similar within species and have differences between the species. However, electrophoresis patterns of serum proteins and brain proteins could be different within the same species. In recent technique like fingerprinting has also got limitations of non-reproducibility of band patterns. Now a day various sophisticated techniques like IEF, PAGE, Immunoblotting, DNA hybridization, PCR, Real time PCR, are also available but they require modern laboratory facilities and trained skills.

REFERENCES

Ashoor, S.H., W.C. Monten and P.G. Stiles, 1998. Liquid chromatographic identification of meats. J. Assoc. Off. Anal. Chem., 71: 397-403.

Bauer, V.C., J. Teifel-Greding and E. Liebhardt, 1987. Species identification of heat denaturized meat samples by DNA analysis. Arch. Lebensmittelhygiene, 38: 149-176.

Borgo, R., C. Soulie-Crosset, D. Bouchon and L. Gomot, 1996. PCR-RFLP analysis of mitochondrial DNA for identification of snail meat species. J. Food Sci., 61: 1-4.

Calvo, J.H., P. Zaragoza and R. Osta, 2001. Random amplified polymorphic DNA fingerprints for identification of species in poultry pate. Poult. Sci., 80: 522-524.

Chikuni, K., T. Tabata, M. Kosugiyama and S. Kato, 1990. Species identification of cooked meats by DNA hybridization assay. Meat Sci., 27: 119-128.

Ebbehoj, K.F. and P.D. Thomsen, 1991. Differentiation of closely related species by DNA hybridization. Meat Sci., 30: 359-366.

Ghatak, S. and J.P.S. Gill, 2009. Molecular techniques for detection and typing of food borne pathogens from livestock products. Proceedings of the Recent Developments in Post Harvest Processing and Value Addition in Livestock Produce, Oct. 22-Nov. 11, CIPHET, Ludhiana, pp: 52-57.

Girish, P.S., A.S.R. Anjaneyulu, K.N. Viswas, B.M. ShivaKumar, M. Anand, M. Patel and B. Sharma, 2005. Meat species identification by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of mitochondrial 12S rRNA gene. Meat Sci., 70: 107-112.

Girish, P.S. and K. Nagappa, 2009. Molecular techniques for meat species identification. Proceedings of the Recent Developments in Post Harvest Processing and Value Addition in Livestock Produce, Oct. 22-Nov. 11, CIPHET, Ludhiana, pp: 57-61.

Govindarajulu, M.C., 1989. Training Report on Meat Speciation Techniques. British Council Technical Co-Operation Training Award, UK., pp: 15-22.

Gracey, J.F., D.S. Collins and R.J. Huey, 1999. Meat Hygiene. 10th Edn., W.B. Saunders Co. Ltd., London.

Janssen, F.W., J.B. Buntjer and J.A. Lenstra, 1998. Species identification in meat by using PCR-generated satellite-DNA probes. J. Ind. Microbiol. Biotechnol., 21: 115-120.

Jones, S.L. and R.L.S. Patterson, 1985. Double antibody ELISA for detection of trace amounts of pig meat in raw meat mixtures. Meat Sci., 15: 1-13.

Kang'ehte, E.K., J.M. Gathuma and K.J. Lindqvist, 1986. Identification of species of origin of fresh, cooked and canned meat and meat products using antisera to thermostable muscle antigens by Ouchterlony's double diffusion test. J. Sci. Food Agric., 37: 157-164.

Kim, H. and L.A. Shelef, 1986. Characterization and identification of raw beef, pork, chicken and turkey meats by electrophoretic patterns of their sarcoplasmic proteins. J. Food Sci., 51: 731-735.

King, N.L., 1984. Species identification of cooked meats by enzyme staining of isoelectrofocusing gels. Meat Sci., 11: 59-72.

Lahiri, D.K., S. Bye, Jr. J.I. Nurnberger, M.E. Hodes and M. Crisp, 1992. A non-organic and non-enzymatic extraction method gives higher yields of genomic DNA from whole-blood samples than do nine other methods tested. J. Biochem. Biophys. Meth., 25: 193-205.

Lawrie, R.A. and D.A. Ledward, 2006. The Structure and Growth of Muscle: Lawrie's Meat Science. 7th Edn., Woodhead Publishing Ltd., Cambridge, England, pp: 42-74, 75-127.

Lee, J.C. and J.G. Chang, 1994. Random amplified polymorphic DNA polymerase chain reaction (RAPD/PCR) fingerprints in forensic species identification. Forensic Sci. Int., 67: 103-107.

Meyer, R., C. Hofelein, J. Luthy and U. Candrian, 1995. Polymerase chain reaction-restriction fragment length polymorphism analysis: A simple method for species identification in food. J. AOAC Int., 78: 1542-1551.

Murray, B.W., R.A. McClymonts and C. Strobeck, 1995. Forensic identification of ungulate species using restriction digestion of PCR-amplified DNA. J. For. Sci., 40: 943-951.

Patterson, R.M. and T.L. Spencer, 1985. Differentiation of raw meat from phlogenically related species by ELISA. Meat Sci., 15: 119-123.

Sachan, N. and V.P. Singh, 2010. Field techniques for meat species specifications. Training Manual of Veterinary Officers of U.P. Govt. Under ATMA Scheme Conducted by Department of Extension DUVASU, Mathura, pp: 63-67.

Sharma, B.D., 1999. Fraudulent Substitution of Meat and its Recognition: Meat and Meat Products Technology (Including Poultry Products Technology). 1st Edn., Japee Brothers Medical Publishers (P) Ltd., New Delhi, pp: 88-94.

Sherikar, A.T., J.B. Khot, B.M. Jayarao and S.R. Pillai, 1988. Use of species specific antesera to adrenal heat -stable antigens for identification of raw and cooked meats by agar gel diffusion and counter immunoelectrophoretic techniques. J. Sci. Food Agric., 44: 63-73.

Singh, V.P., 2008a. Laboratory Manual of Meat and Meat Products Technology (Including Poultry Products Technology. DUVASU, Mathura, pp: 1-6.

Singh, V.P., 2008b. Training Booklet for Internship Students on Field Oriented Issues of Livestock Products Technology. DUVASU, Mathura, pp: 20-23.

Singh, V.P. and N. Sachan, 2009. Laboratory Manual of Abattoir Practices and Animal By-products Technology. DUVASU, Mathura, pp: 25-35.

Singh, V.P., 2010. Livestock technology and service: Internship manual as per VCI syllabus. College of Veterinary Science and Animal Husbandry, Pt. D.D.U. Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura, pp: 136-153.

Singh, V.P. and N. Sachan, 2010. Collection and dispatch of meat samples for vetrolegal cases. Training Manual of Veterinary Officers of U.P. Govt. under ATMA Scheme, Department of Extension DUVASU, Mathura, pp: 63-67.

Skarpeid, H.J., K. Kvaal and K.I. Hildrum, 1998. Identification of animal species in ground meat mixtures by multivariate analysis of isoelectric focusing protein profiles. Electrop Horesis, 19: 3103-3109.

Thornton, H., 1968. Textbook of Meat Inspection. 6th Edn., Tindall and Cassel, Bailliere, London.

Wintero, A.K., P.D. Thomsen and W. Davies, 1990. A comparison of DNA-hybridization, immunodiffusion, countercurrent immunoelectrophoresis and isoelectric focusing for detecting the admixture of pork to beef. Meat Sci., 27: 75-85.