

Nuclear and mitochondrial DNA markers in traceability of retail beef samples¹

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ABSTRACT.- Cesar A.S.M., Biase F.H., Ripamonte P., Luchiarri Filho A., Merighe G.K. & Meirelles F.V. 2010. **Nuclear and mitochondrial DNA markers in traceability of retail beef samples.** *Pesquisa Veterinária Brasileira* 30(9):783-786. Laboratório de Morfofisiologia Molecular e Desenvolvimento, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Av. Duque de Caxias Norte 225, Pirassununga, SP 13635-900, Brazil. E-mail: meirelff@usp.br

Several characteristics are important in a traceability system of animal products, such as age at slaughter, breed composition, besides information of the productive chain. In general, the certification agent records information about the animals and the system which it came from, although cannot guarantee that the slaughtering, meat processing and distribution are error proof. Besides, there is a differential price, at least at the international market, based on sex and breed composition of the animals. Genetic markers allow identification of characteristics controlled in the beef cattle traceability program, as sex and breed composition, in order to correctly identify and appraise the final product for the consumer. The hypothesis of this study was that the majority beef samples retailed in the local market originate from female with a great participation of zebu breeds. Therefore, the objective of this work was to characterize retail beef samples with DNA markers that identify cattle sex and breed composition. Within 10 beef shops localized in Pirassununga, SP, Brazil, 61 samples were collected, all were genotyped as harboring *Bos taurus* mitochondrial DNA and 18 were positive for the Y chromosome amplification (male). For the marker sat1711b-*Msp*I the frequency of the allele A was 0.278 and for the marker Lhr-*Hha*I the frequency of the allele T was 0.417. The results of sat1711b-*Msp*I and Lhr-*Hha*I allelic frequencies are suggestive that the proportion of indicus genome compared with the taurine genome in the market meat is smaller than the observed in the Nellore breed. The procedure described in this study identified sex and subspecies characteristics of beef meat samples, with potential application in meat products certification in special as an auxiliary tool in beef cattle traceability programs.

INDEX TERMS: *Bos indicus*, beef, stDNA, mtDNA, traceability.

RESUMO.- [Marcadores de DNA nuclear e mitocondrial para rastreabilidade da carne bovina comercializada.]

Várias características são importantes no sistema de rastreabilidade, como o sexo, a idade, a raça e/ou a composição racial dos animais, além de dados da cadeia produtiva. Em

geral, a empresa certificadora dispõe das informações do animal que está sendo abatido, porém não tem condições de garantir se houve erro entre abate, desossa, processamento e a distribuição dos produtos. Existe diferenciação no custo e na qualidade dos produtos cárneos, especialmente no mercado internacional, em virtude do sexo e composição racial dos animais. Os marcadores genéticos permitem identificar as características que são controladas num programa de rastreabilidade bovina tais como sexo e composição racial, permitindo identificar e avaliar corretamente para o consumidor, o produto final. A hipótese deste estudo foi que a maioria das amostras de carne bovina vendida no mercado local seria proveniente de fêmeas e com grande participação de raças Zebu. O objetivo deste trabalho foi caracterizar amostras de

¹ Received on April 7, 2010.

Accepted for publication on July 15, 2010.

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carne bovina com marcadores de DNA para identificar o sexo e a composição racial. Em dez pontos comerciais da cidade de Pirassununga, SP, Brasil, foram coletadas 61 amostras e todas foram genotipadas como possuindo DNA mitocondrial *Bos taurus* e 18 foram positivos para amplificação do cromossomo Y (macho). Para o marcador sat1711b-*Msp* I a frequência alélica do A foi 0.278 e para o marcador Lhr-*Hha* I a frequência alélica do T foi 0.417. Os resultados das frequências alélicas do sat1711b-*Msp* I e Lhr-*Hha* I apresentaram menor proporção do genoma *Bos indicus* em relação ao *Bos taurus* quando comparado ao rebanho Nelore. Com a metodologia descrita neste trabalho foi possível avaliar o sexo e as características de subespécie das amostras de carne bovina, tendo uma importante aplicação para a certificação de produtos cárneos especialmente, em programas de rastreabilidade animal.

TERMOS DE INDEXAÇÃO: *Bos indicus*, carne bovina, stDNA, mtDNA, rastreabilidade.

INTRODUCTION

After the spreading of foot and mouth disease, BSE and other sanitary problems around the world, the European Community countries, as well as countries from other regions started a demand for traceability of animal origin products (Daqq et al. 2005). Traceability means the capability of tracing back and forward with confidence all the steps of a product and its production process (Vitorelli Neto 2004).

Brazil has the second largest beef herd in the world (FAO 2007), with its increasing levels of efficiency and productivity therefore keeping high standards is vital to the beef cattle industry. In this scenario, traceability is an important tool for beef chain in order to maintain and to open new beef markets.

In the traceability system, several characteristics are controlled as, sex, subspecies and the degree of crossbreeding of the animals, especially when zebu cattle (*Bos indicus*) is part of the crossbreeding scheme (Comunidades Europeias 2000). Although consistent procedures are applied on a traceability program, no system can be fail proof unless checking procedures can be performed.

The cattle can be divided in two main groups: the European (*B. taurus*) and the Indian zebu cattle (*B. indicus*). Although the classical division by Lineu considers these groups as separate species, several authors have stated that they are subspecies (Manwell & Baker 1980).

Cattle *B. taurus* breeds such as Angus and Hereford now became synonymous of the most appreciated and tasty flesh of the world (Luchiari & Mourão 2006), highly priced in the international market. The Japanese market, for example, prefers beef with a high percentage of intramuscular fat. It can reach 20 to 30% of fat in the superior quality of beef type (Jurie et al. 2007), with a typical characteristic of these breeds. Thus, animals that have in their composition *B. taurus* breeds can reach the higher prices market. Increased demand for these products is seen in the Asian markets for beef.

Molecular markers have been used for a precise identification in between these groups, the European (*B. taurus*) and the

Indian zebu cattle (*B. indicus*). According to Loftus et al. (1994) DNA polymorphism sequence of mitochondrial (mtDNA) was identified in D-loop region, as well as in less variable regions as transporter RNAs (Pegoraro et al. 1995) and 16S (Meirelles et al. 1999). A mendelian inherited variation was also described in the centromeric satellite region of the nucleic DNA which introduces a restriction site in part of the *B. indicus* animals (Nijman et al. 1999; Ripamonte 2002). These sequences usually localized at the chromosomes heterochromatin are close to the centromeres (Charlesworth et al. 1994).

Analyzing the 1711b DNA satellite of *B. taurus*, *B. indicus* and its hybrids, it was observed that species closely related can show variable sequences of these satellites, which can be used as marker in crossbreed studies of zebu with european cattle breeds. Actually, several markers are polymorphic recognized between *B. taurus* and *B. indicus* animals. The gene of the luteinizing hormone receptor (LHR) contains these polymorphisms. A punctual mutation in the 1328 position which carries to a base change, cytosine (C) for thymidine (T) was described in Nelore breed (Milazzotto 2007). Zebu cattle specifically harbor the T allele (0.755 for allele T versus 0.245 for allele C), instead of European animals that harbors C allele exclusively. The recent report of the bovine genome assembly (Elsik et al. 2009), as well as the study identifying variability among cattle from different breeds will provide new features about the cattle genome that will certainly be useful for traceability programs. The hypothesis of this study was that the majority beef samples retailed in the local market originate from female with a great participation of zebu breeds.

Based on these evidences, the objective of this study was to characterize beef samples with DNA markers to identify the gender and racial composition.

MATERIALS AND METHODS

Beef samples (n=61) were collected in ten different selling points in Pirassununga, SP, Brazil, along the months of April and May of 2005 and DNA from all samples was extracted by salt precipitation (Biase et al. 2002). All samples were informed to be male derived. Briefly, about 0,2g of muscle were weighed, pondered with liquid nitrogen and dissolved in 800µL of lyses buffer and 4µL of protein k solution (50mg/µL). The cell lysate solution was heated 55°C for 5 h. After addition of 300 µL of salt saturated solution (NaCl 5M), we proceeded centrifugation at 21.000xg for 15 minutes. The supernatant was collected in a new tube and 1.2mL of ethanol 100% was added to induce DNA precipitation. The pellet was eluted in 50µL of DNase free water. DNA amount was estimated by spectrophotometry at 260/280nm absorbance and quality observed in agarose gel (0.8%) electrophoresis stained with ethidium bromide. Primers and restriction enzyme used in this article are listed in Table 1. The SRY marker identifies the male sample origin by the presence or not of the Y chromosome amplified fragment. This reaction was run in a multiplex with a satellite region (Ellis & Harpold 1986; Ellis & Harpold 1988) common to both sex genomes. The reaction was set with 120ng of DNA, enzyme buffer (1x), MgCl₂ (50mM), dNTP (100µM each), 10 pmol of each primer pair and Taq DNA polymerase (1U) in a final volume of 25µl. Thermocycler program was 95°C, 2

Table 1. Primers and restriction enzyme used for each polymorphic loci

Marker	Primers 5' → 3'	Restriction enzyme
mtDNA	F - CCC AAC GAG GAA AAT ATA CC R - AAC CGC AAA CAA CCT CTT CC	<i>Hind</i> III
Sat1711b	F - CCC ACT ACC TCT CTC TGA AAA R - TGA TCC AGG GTA TTC GAA GGA	<i>Msp</i> I
Lhr	F - CAA ACT GAC AGT CCC CCG CTT T R - CTC CGA GCA TGA CTG GAA TGG C	<i>Hha</i> I

minutes; 35x (94°C, 25 seconds; 62°C, 20 seconds; 72°C, 45 seconds) and a final heat of 72°C, 10 minutes. The LHR and sat1711a were amplified in order to identify the presence of nuclear specific *B. taurus* and *B. indicus* alleles, respectively. Hence, in order to identify the matrilineal ancestrally a mitochondrial SNP (mtDNA) was also evaluated. The reactions were carried individually and the reaction conditions were as described above, except for the annealing temperature: 58°C, 62°C and 58°C for LHR, sat1711a and mtDNA primers, respectively. Reactions products for LHR, sat1711a and mtDNA (15µl) were directly subjected to enzymatic restriction (*Hha* I, *Msp* I and *Hind* III, respectively). The final products of PCR or PCR-RFLP were submitted to electrophoresis in 2% agarose gel with ethidium bromide. The images were captured in a laser scanner FujiFilm3000.

Genotypic and allelic frequencies were calculated for the entire sample and separately for male and female samples (Weir & Cockerham 1984). The allelic frequencies were compared between male and female using Fisher exact test in contingency table (Raymont & Rousset 1995) and the probability of genotypic disequilibrium between each polymorphic markers were estimated in the sample and within male and female samples (Guo & Thompson 1992). Comparisons between the allelic data observed herein and the previously reported was performed using contingency table applying Fisher's method (Raymont & Rousset 1995). Estimates were calculated using the GENEPOP software (Raymont & Rousset 1995) and a result was considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Amplification of the four markers was possible for all samples. The observed PCR or PCR-RFLP bands profiles are depicted in Figure 1.

Of the 61 collected samples, 39 (64%) were positive for the Y chromosome amplification (male) and only 27 were

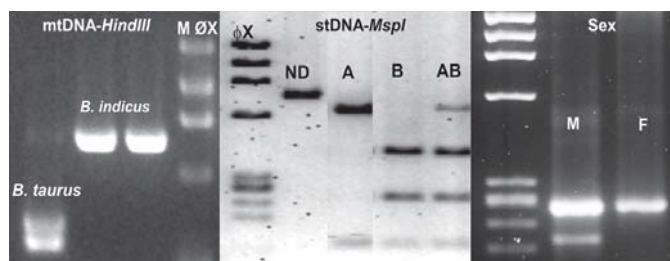


Fig.1. Electrophoresis on agarose gel 2% representing PCR-RFLP of mtDNA - *Hind* III, stDNA - *Msp* I, and sexing markers respectively.

Table 2. Genotypic frequencies for mitochondrial DNA (mtDNA-*Hind* III) and gene frequencies for satellite 1711b-*Msp* I and LHR-*Hha* I polymorphisms

	N	mtDNA		Sat1711b		Lhr	
		<i>B. indicus</i>	<i>B. taurus</i>	A	B	C	T
Male	18	0.0	1.0	0.278	0.722	0.583	0.417
Female	09	0.0	1.0	0.111	0.889	0.444	0.556
Total	27	0.0	1.0	0.222	0.778	0.537	0.463

genotyped as harboring *B. taurus* mitochondrial DNA and used in this experiment. The allelic frequencies of the LHR and sat1711b markers for total sample and within the sexes are presented in (Table 2). Using the satellite marker, one individual was genotyped as AA (*B. indicus* specific allele) and 10 were heterozygous. The LHR marker evaluation showed most of the individuals with heterozygous genotype (n=15) and 07 animals were genotyped as homozygous for the *B. taurus* specific allele (C). Both LH and sat1711b alleles were in Hardy-Weinberg equilibrium and there was no genotypic disequilibrium between the sat1711b and LHR loci.

Most of the Brazilian heard is comprised of Nelore breed or Nelore crossbred animals. Despite the limited sample number, our results compare with Nelore data and observed that the mitochondrial DNA genotype and nuclear allelic frequencies observed herein are not in accordance with previous findings observed for Nelore sample ($p < 0.05$). Meirelles et al. (1999) reported the presence of indicus mtDNA in 43.4% of the purebred Nelore sample examined. This data however may over estimate *B. indicus* mtDNA contribution to the Nelore population since it included closed herds with descendent exclusive from imported animals. Overall data from Nelore sample (n= 367) genotyped in our laboratory indicate that 16.34% have *B. indicus* matrilineal origin (Ripamonte 2002). Regarding the satellite polymorphism, the A allele frequency in the laboratory dataset (Nelore, n= 311) was 0.4068 (Ripamonte 2002). Milazzoto (2007) reported LHR-*Hha* I allelic estimation for Nelore breed and the C allele frequency was 0.245.

Nelore is the zebu breed most abundant in the Brazilian heard, however the results of sat1711b-*Msp* I and LHR-*Hha* I allelic frequencies are suggestive that the proportion of indicus genome compared with the taurine genome in the market meat is smaller than the observed in the Nelore purebred herd. The taurine matrilineal origin of the slaughtered animals, when compared with data of purebred animals reported previously by Ripamonte (2002) is in accordance with this observation. Further analysis increasing the number of markers and animals genotyped will be helpful to estimate the genomic composition of the meat, allowing the estimation of subspecies contribution at individually and therefore providing parameters that can help the establishment of traceability programs.

CONCLUSIONS

Based on these results we conclude that beef marketed in Pirassununga-SP are predominantly from males with the

Bos indicus specific alleles frequency representatively lower compared to Nellore cattle.

With the procedure described it was possible to evaluate sex and subspecies contribution to meat samples. These methodologies have potential application in meat products certification in special as an auxiliary tool in beef cattle traceability programs.

Acknowledgements.- To FAPESP and CNPq for the financial support.

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