PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research

ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 38(11):2052-2055, novembro 2018
DOI: 10.1590/1678-5150-PVB-5963

Original Article
Animais de Produção/*Livestock Diseases*

Prevalence of mutations responsible for glycogen storage disease type-II and congenital myasthenic syndrome in Brazilian Brahman cattle[1]

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ABSTRACT.- Trecenti A.S., Fernandes A.V., Andrade D.G.A., Pimenta-Oliveira, A. Borges A.S. & Oliveira-Filho J.P. 2018. **Prevalence of mutations responsible for glycogen storage disease type-II and congenital myasthenic syndrome in Brazilian Brahman cattle.** *Pesquisa Veterinária Brasileira 38(11):2052-2055*. Departamento de Clínica Veterinária, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Rua Prof. Dr. Walter Mauricio Correa s/n, Cx. Postal 560, Botucatu, SP 18618-681, Brazil. E-mail: jose.oliveira-filho@unesp.br

Glycogen storage disease type II (GSD-II) and congenital myasthenic syndrome (CMS) are important autosomal recessive disorders in Brahman cattle. The objective of this study was to investigate the presence of mutations responsible for GSD II (E7, c.1057_1058delTA; and E13, c.1783C>T) and CMS (c.470del20) in purebred Brazilian Brahman cattle and in purebred Brahman bulls that were routinely used in breeding programs in Brazil. A total of 276 purebred Brahman cattle (167 females and 109 males, with ages ranging from 12-24 months) and 35 frozen semen samples taken from purebred Brahman bulls (22 bulls from the USA, 11 Brazilian bulls, one Argentine bull and one Australian bull) were used in this study. Genomic DNA was purified from hair root samples and from semen samples. Purified DNA was used in PCR genotyping to mutations c.1057_1058delTA (E7) and c.1783C>T (E13) in the *GAA* gene and c.470del20 in the *CHRNE* gene. The PCR products were purified and sequenced. The genotypic frequencies per polymorphism were estimated separately. Of the 276 Brahman cattle tested, 7.3% were identified as heterozygous for E7. All Brahman cattle studied were homozygous for the wild-type E13 allele. The E7 mutations was identified as heterozygous in 8.6% (3/35) of the commercial semen samples, whereas the E13 mutations was not identified. The c.470del20 mutation was identified as heterozygous in 0.73% of the hair root samples, but this mutation was not present in any semen sample assessed. No study had previously evaluated the prevalence of mutations responsible for GSD II or CMS in Brazilian Brahman cattle. In summary, the E7 and c.470del20 mutations are present in the Brazilian Brahman herd, and control measures should be adopted to prevent an increase in the incidence of GSD-II and CMS in Brahman cattle in Brazil.

INDEX TERMS: Mutation, glycogen storage disease, congenital myasthenic syndrome, Brazilian Brahman cattle, generalised glycogenosis, Pompe disease, Brazil.

RESUMO.- [**Prevalência de mutações responsáveis pela estocagem de glicogênio tipo II e síndrome miastênica congênita em bovinos brasileiros da raça Brahman.**] A doença de armazenamento de glicogênio tipo II (DAG-II) e a síndrome miastênica congênita (SMC) são importantes doenças autossômicas recessivas no gado Brahman. O objetivo deste estudo foi investigar a presença das mutações responsáveis pela DAG-II (E7, c.1057_1058delTA; e E13, c.1783C>T) e pela SMC (c.470del20) em bovinos da raça Brahman e em touros Brahman que são rotineiramente utilizados em programas de reprodução no Brasil. Um total de 276 amostras de bulbo piloso de bovinos Brahman (167 fêmeas e 109 machos, com idade variando de 12 a 24 meses) e 35 amostras de sêmen congeladas de touros Brahman (22 touros americanos, 11 touros brasileiros, um touro argentino e um touro australiano) foram usados neste estudo. O DNA genômico foi purificado, das amostras de bulbo

[1] Received on May 14, 2018.
Accepted for publication on May 21, 2018.
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piloso e de sêmen, e utilizado na genotipagem por PCR das mutações c.1057_1058delTA (E7) e c.1783C>T (E13) no gene GAA e c.470del20 no gene CHRNE. Os produtos de PCR foram purificados e sequenciados. A frequência genotípica para cada polimorfismo foi estimada separadamente. Dos 276 Brahman testados, 7,3% foram identificados como heterozigotos para E7. Todos os Brahman foram homozigotos wild-type para o alelo E13. A mutação E7 foi identificada em homozigose em 8,6% (3/35) das amostras de sêmen comerciais, enquanto que a mutação E13 não foi identificada. A mutação c.470del20 foi identificada em heterozigose em 0,73% das amostras de bulbo piloso, mas esta mutação não estava presente nas amostras de sêmen avaliadas. Nenhum estudo prévio avaliou a prevalência das mutações responsáveis pela DAG-II ou SMC em bovinos Brahman brasileiro. Em suma, as mutações E7 e c.470del20 estão presentes no rebanho Brahman brasileiro, e medidas de controle devem ser adotadas para prevenir o aumento da incidência da DAG-II e SMC em bovinos da raça Brahman no Brasil.

TERMOS DE INDEXAÇÃO: Mutação, glicogênio, síndrome miastênica congênita, bovinos, raça Brahman, glicogenose generalizada, doença de Pompe, Brasil.

INTRODUCTION

Genetic disorders affecting breeds of cattle are relatively common and may be economically important in particular herds (Jolly 2002). Brazil is an important country for cattle breeding for milk and meat, having one of the largest herds in the world. Brazilian livestock production is characterised by large-scale extensive operations under grazing conditions using mainly natural mating or artificial insemination (AI) as reproductive strategies, although the use of in vitro embryo production (IVP) has increased each year for animals of high genetic value (Pimenta-Oliveira et al. 2011). In contrast, there have only been a few studies of genetic diseases in the Brazilian cattle herd.

The glycogen storage disease type II (GSD-II), also known as generalised glycogenosis or Pompe's disease, and congenital myasthenic syndrome (CMS) are important autosomal recessive disorders (Dennis et al. 2002, Thompson et al. 2003a). The GSD-II in Brahman cattle is caused by two independent mutation, c.1057_1058delTA (E7) and c.1783C>T (E13), in the gene coding the acidic α-glucosidase (GAA) enzyme (Dennis et al. 2000). Animals homozygous for any of these two polymorphisms will produce a truncated GAA, resulting in glycogen accumulation in lysosomes. Affected individuals show progressive muscle weakness, problems with motor coordination and abnormal growth (O'Sullivan et al. 1981, Reichmann et al. 1993). Usually, this condition is progressive and animals die before 12 months of age (Zlotowski et al. 2006).

Congenital myasthenic syndrome (CMS) in Brahman cattle is caused by a homozygous 20 base pair deletion (c.470del20) in the gene coding for the epsilon subunit of the acetylcholine receptor (CHRNE) (Kraner et al. 2002). This polymorphism, when homozygous, results in a non-functional allele and the inability to produce functional adult-type nicotinic acetylcholine receptor. This, in turn, results in progressive severe muscle weakness observed either at birth or within the first month of life, which leads to death shortly thereafter (Thompson 1998, Thompson et al. 2003b).

Brahman cattle is a cosmopolitan breed, found in more than 70 countries around the world. In Brazil, the breed was mainly derived from American Brahmans, being officially introduced in Brazil in 1994. Currently, this breed is the second major meat breed of zebuine origin in Brazil. Although the Brazilian Brahman herd is considered the third largest Brahman herd in the world, unlike in other countries (Dennis et al. 2002, Thompson et al. 2003a), no study has previously evaluated the prevalence of mutations responsible for GSD-II or CMS in Brazilian Brahman cattle. Therefore, the objective of this study was to investigate the mutation E7, E13 and c.470del20 in Brazilian Brahman cattle and commercial Brahman semen used in Brazil.

MATERIALS AND METHODS

Ethics statement. All procedures were approved by the Board of Ethics and Animal Experimentation of the institution (Protocol no. 048/2016 - CEUA).

Experimental samples. A total of 276 hair root samples were collected from purebred Brahman cattle (167 females and 109 males, with ages ranging from 12-24 months) from meat farms located in four geopolitical regions of Brazil, North+Midwest (28/276), Southeast (136/276) and Southern (112/276), under a strict confidentiality agreement to ensure the anonymity of establishments, owners and animals. The sampling was randomly performed by ABCZ technicians (Associação Brasileira dos Criadores de Zebu) during the certification of animals by ABCZ in farms. In addition, 35 frozen semen samples taken from purebred Brahman bulls (22 bulls from the USA, 11 Brazilian bulls, one Argentine bull and one Australian bull) and obtained from reproduction companies in Brazil were also used in this study.

DNA purification and genotyping analysis. Genomic DNA was purified from hair root samples using an in-house method and from semen samples using the GenElute™ Genomic Blood DNA Kit (Sigma-Aldrich®) according to the manufacturer's instructions. The DNA obtained was used to genotype the mutations c.1057_1058delTA (E7) and c.1783C>T (E13) in the GAA gene and c.470del20 in the CHRNE gene. Two specific primer pairs were designed with Primer Express software (Applied Biosystems, Grand Island, NY) to genotype the mutations E7 (258-bp) (CGGGATCCTGGACGTGTA, AGTAGGCCCTGGTCATATTCT), and E13 (323-bp) (AGCTATCCGGTCCTTGA, GACCTGGCTCTGGACAAAC) in the *GAA* gene. The specificity of the PCR primers was evaluated in silico with the Basic Local Alignment Search Tool (National Center for Biotechnology Information/USA). A previously described specific primer pair (Thompson et al. 2003b) was used to amplify a 211-bp fragment containing the polymorphism c.470del20 in the *CHRNE* gene. Purified DNA from the hair root and semen samples was used in PCR genotyping. Polymerase chain reactions (25μL) contained 12.5μL of GoTaq Green PCR Master Mix (Promega, Madison, WI), 0.3μM of each forward and reverse primer, 2.5μL of template DNA, and nuclease-free water up to the final volume. The amplification conditions were as follows: initial denaturation at 95°C for 2 minutes; followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 62°C (c.1057_1058delTA and c.1783C>T) or 60°C (c.470del20) for 30 seconds, and extension at 72°C for 1 minute; followed by a final extension at 72°C for 5 minutes. Amplicons were analysed by 1.5% agarose gel electrophoresis, purified, and subjected to direct sequencing.

Sequencing analysis. To sequence the DNA, 10μL of each PCR product, 5μL of the purified forward primer and the BigDye® Terminator Cycle Sequencing Kit were used (Life Technologies™, CA,

USA). The sequences were determined using the ABI 3500 Genetic Analyzer (Life Technologies™ CA, USA). The obtained sequences and the electropherograms were analysed using Sequencher™ 5.1 (Gene Codes, MI, USA). The sequences were compared with the normal *Bos taurus GAA* or *CHRNE* gene sequences using BLAST (Basic Local Alignment Search Tool, http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Data analysis. The genotypic frequencies per polymorphism were estimated separately. Prevalence estimates provided the basis for calculation of the number of heterozygous and homozygous recessive calves born annually, assuming random mating (Thompson et al. 2003a).

RESULTS

Hair root samples were collected during the year of 2,014 and all animals were apparently healthy, and no clinical signs of GSD-II or CMS were observed in animals during the sampling procedures. Of the 276 Brahman tested, 20 animals (7.3%; 10 females and 10 males) were identified as heterozygous for the E7 polymorphism (E7/N) and 256 (92.7%) were homozygous for the wild-type allele (N/N). The E7 polymorphism was observed as heterozygous in 9.6% (13/136) of the tested animals from the Southeast region, 5.4% (6/112) from the Southern region and 3.6% (1/28) from the North+Midwest regions. All Brahman cattle studied were homozygous for the wild-type allele E13. Two (0.73%) Brahman cattle were identified as heterozygous for the c.470del20 polymorphism, including one female from the Southern region and one male from the Midwest region. The other 274 (99.3%) Brahman cattle were homozygous for the wild-type allele of this polymorphism. No animal was found to be heterozygous for both polymorphism (E7 and c.470del20).

Considering the E7 and c.470del20 mutation frequencies and random mating, may be estimated that 7,034 E7 heterozygous calves and 727 c.470del20 heterozygous calves will be born in every 100,000 births, and 133 E7-affected calves and one c.470del20-affected calf will be born in every 100,000 births in Brazil.

Of the 35 semen samples assessed, three (8.6%) were identified as heterozygous to E7, including two bulls from the USA and one bull that was born in Brazil but was the son of parents imported from the USA. The E13 c.470del20 polymorphism was not identified in the studied bulls.

DISCUSSION

Glycogen storage disease type II and CMS are important autosomal recessive disorders in Brahman cattle herds from Australia (Dennis et al. 2002, Thompson et al. 2003a) and South Africa (Thompson et al. 2003a, 2007), and although the origin of these animals and the Brazilian herd are the same, the prevalence of these diseases in Brazil was unknow. In this study, we used purebred Brahman cattle registered with ABCZ, as the bulls were frequently used in several breeding programmes for beef cattle in Brazil and in other countries.

In the present study, the frequency of animals heterozygous for E7 GSD-II was similar to South Africa (7.3%) (Thompson et al. 2003a); and lower than Australia (12%) (Dennis et al. 2002). Assuming random breeding over the Australian Brahman herd, approximately 400 affected calves will be born for every 100,000 births. Because GSD-II is a fatal disease, the annual loss was predicted in 2002 to be USD $1,035,000 (Dennis et al. 2002), and proportional losses might also occur in Brazil. Unfortunately, these losses may be greater in both Australia and in Brazil because the standard practice of line breeding increases inbreeding, leading to an inevitable increase in the frequency of homozygous recessive alleles (Dennis et al. 2002).

The prevalence of E7 carriers was higher in the Southeast region than in the Southern and North+Midwest regions. Although the southeast region has the highest number of purebred Brahman cattle registered in ABCZ, animals heterozygous for E7 were found in all Brazilian regions studied, indicating that this polymorphism is widespread throughout Brazil. On the other hand, although the E13 GSD-II mutation provokes generalised glycogenosis in Australian Brahman cattle, it is less common and was restricted to descendants of one bull imported from the USA, which had arrived in Australia in 1982 (Dennis et al. 2002). This is probably why the 1783T allele was not detected in Brazilian Brahman cattle in the present study.

In the present study, the two animals identified as c.470del20 carriers were from two farms located in the Southern and Midwest regions of Brazil. The frequency of the c.470del20 carriers in Brazil was similar to South Africa (Thompson et al. 2003a). This low frequency probably does not have a significant economic impact on the Brazilian Brahman herd. However, because c.470del20 carriers are heavier at 600 days of life compared to wild-type animals, there is a selective advantage that tends to increase the frequency of the mutant allele in the population if the genotypes of bulls and/or cows are unknown (Thompson et al. 2007). This human interference may lead to artificial selection resulting in economic losses that may be difficult to compensate (Ciepłoch et al. 2017).

The 24 imported bulls together accounted for 50,000 descendants registered in ABCZ since the introduction of the breed in Brazil (1994-2014). The two American bulls identified as E7 carriers together produced 3,000 registered offspring in the same period. Unfortunately, data about the number of descendants of the 11 Brazilian bulls used in the present study was not available. However, all commercial semen samples assessed in the present study, including the 11 Brazilian bulls, belonged to bulls that were routinely used in breeding programmes in Brazil.

Calves affected by GSD-II usually have progressive muscular weakness and ill-thrift, with a life expectancy of less than 12 months (Reichmann et al. 1993). Only one report of E7 GSD-II was described in a farm located in the Southern region of Brazil (Zlotowski et al. 2005). However, the data of the present study, reinforce the hypothesis that affected animals (E7 GSD-II) are probably being born in Brazil and dying before weaning without having the cause of death correctly diagnosed. Additionally, non-detection of the alleles of the other two polymorphisms (E13 and c.470del20) in the cohort of the assessed bulls may allude to the lower relevance of these two mutations for the Brazilian Brahman herd compared to E7 mutation.

CONCLUSION

The E7 and c.470del20 mutations are present in Brazilian Brahman herd, and control measures, as have been adopted in Australia, should be adopted in Brazil to prevent an increase in the incidence of GSD II and CMS in Brazilian Brahman cattle.

Conflict of interest statement. - The authors declare that there are no conflicts of interest.

Acknowledgments.- This study was carried out at the São Paulo State University (Unesp) and in part as fulfilment of the master science thesis of AST at São Paulo State University (Unesp), Brazil. The authors also thank Associação Brasileira dos Criadores de Zebu for collecting the samples. This study was supported by a grant from the CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - AUX-PE-PREMIO - 432/2014) and by a grant from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (scholarship to AST).

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