DNA MICROSATELLITES FOR GENETIC IDENTIFICATION IN BRAZILIAN MURRAH WATER BUFFALOES

[*Uso de Microssatélites para identificação genética de búfalos brasileiros da raça Murrah*]

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ABSTRACT - Brazilian water buffalo (*Bubalus bubalis*) population is currently approximately 3,000,000. Despite of this fact, genealogical control is still one of the problems of the Brazilian selection and breeding programs. The DNA test is important to develop a system that allows the animal genealogy certification as well as its undeniable individual and parentage identification. The present study was performer by using a panel of 14 microsatellites markers in Brazilian Murrah buffaloes (n=100) in order to estimate the genetic variability and calculate the parentage exclusion probability. A total of 92 alleles were detected in the whole sample and the number of alleles varied from one (locus D5S2) to 13 (locus CSSM47). The Polymorphism Information Content values ranged from 0.00 (locus D5S2) to 0.845 (locus BM1706). Heterozygosity values ranged from 0.00 (D5S2) to 0.861 (BM1706). The paternity exclusion probabilities when only one and both parents were analyzed was 0.985424 (PE-1) and 0.999541 (PE-2), respectively. Observed a probability of exclusion of 0.999998% when both parents (PE-3) were tested for the set of 14 microsatellites. This panel is already used in Italy for the Mediterranean buffaloes, one of the four breeds raised in Brazil. However, it is highly recommended that new loci are analyzed in order to increase the microsatellites panel repertoire used for genealogical studies.

Keywords: *Bubalus bubalis*, individual and parentage identification.

RESUMO – Atualmente, o tamanho do rebanho bubalino brasileiro (*Bubalus bubalis*) é de cerca de 3.000.000. Apesar deste fato, o controle genealógico ainda é um dos problemas em programas brasileiros de seleção e de melhoramento animal. O teste de DNA é importante para que se possa desenvolver um sistema que permita a certificação genealógica, bem como as inegáveis identificações individuais e de paternidade. O presente estudo teve como objetivo avaliar um painel de 14 marcadores microssatélites em búfalos brasileiros da raça Murrah (n=100), a fim de se estimar a variabilidade genética, probabilidade de exclusão de parentesco. Foram detectados 92 alelos em toda a amostragem, sendo que o número de alelos variou de um (*locus* D5S2) a 13 (*locus* CSSM47). Os valores do Contepudo de Informação Polimórfica variaram de 0,00 (*locus* D5S2) a 0.845 (*locus* BM1706). Os valores da Heterozigosidade variaram de 0,00 (*locus* D5S2) a 0.861 (*locus* BM1706). As probabilidades de exclusão de paternidade quando apenas um e ambos os pais foram analisados foram de 0,985424 (PE-1) e 0,999541 (PE-2), respectivamente. Foi observada probabilidade de exclusão de 0,999998 quando ambos os pais foram testados (PE-3) para o conjuntos dos 14 microssatélites. Este painel já é utilizado na Itália para búfalos, da raça Mediterrâneo, uma das quatro raças criadas no Brasil. No entanto, é altamente recomendável que novos *loci* sejam analisados a fim de aumentar o painel de microssatélites para estudos genealógicos.

Palavras-Chave: *Bubalus bubalis,* identificação individual e de paternidade.

INTRODUCTION

Buffaloes (*Bubalus bubalis*) were introduced in Brazil, Marajó Island, Pará, from the nineteenth century from Asia, Europe (Italy) and the Caribbean (Bernardes, 2007). The great ability of adaptation to various environments, the high fertility and the longevity in production, allowed the herd to evolve. The initial 200 animals into the country resulted in increasing the herd to about three millions heads (ABCB, 2010). Despite of this fact, genealogical control is still one of the weaknesses selection and

breeding programs in Brazilian buffaloes. The DNA test is important to develop a system that allows animal genealogy certification as well as its undeniable individual and parentage identification. Many authors have used microsatellites for molecular genetics characterization of buffalo (Coletta *et al*., 2010; Muraleedharan *et al*., 2009; Nagarajan *et al*., 2009; Elbeltagy *et al*., 2008; Vijh *et al*., 2008; Zhang *et al*., 2007; Kumar *et al*., 2006; Navani *et al*., 2002; Moioli *et al*., 2001; Ritz *et al*., 2000) but only few published papers have included molecular markers in Brazilian populations analysis (Rogberg-Muñoz *et al*., 2010; Albuquerque *et al*., 2005).

The aim of this study is to validate a panel of 14 microsatellites markers as a tool of genealogical certification of Brazilian Murrah buffaloes in order to estimate the genetic variability and calculate the exclusion power and the match probability.

MATERIALS AND METHODS

DNA of 100 Brazilian Murrah buffaloes was extracted from hair follicles, consisting of distinct groups of related individuals and unrelated individuals from the states of Minas Gerais, Pará and São Paulo. For DNA extraction by hair follicles was used the technique described by Veterinary Genetics Laboratory at the University of California-Davis, modified by Yves Amigues, INRA, Jouy-en-Josas.

Samples were amplified using a panel of 14 microsatellite markers (BMC1013, BM1706, BM922, CSSM19, CSSM38, CSSM42, CSSM47, CSSM60, CYP21, INRA26, INRA6, MAF65, RM4 and D5S2) recommended by ISAG/2010 – *International Society by Animal* Genetics/2010. After electrophoresis of PCR products performed by automatic DNA analyzer ABI Model 3130 (AppliedBiosystems), analysis were made using GeneMapper program.

The statistical analysis was performed using software developed by the Stormont Laboratories, Inc- Woodland (UCDavis - CA – USA, 1997), for use under license, expressed in base pairs (bp), representing the specific genotyping. This software estimated parentage exclusion probability (PE), polymorphism information content (PIC) and heterozygosity values (Het.).

RESULTS AND DISCUSSION

A total of 92 alleles were detected in the whole sample and the number of alleles varied from one (locus D5S2) to 13 (locus CSSM47). The PIC values ranged from 0.00 (locus D5S2) to 0.845 (locus BM1706). Heterozygosity values ranged 0.00 (D5S2) to 0.861 (BM1706). Paternity exclusion probabilities ranged from 0.985424 (PE-1) and 0.999541 (PE-2) when only one and both parents were analyzed respectively. The probability of exclusion of both parents (PE-3) reached 0.999998% for the set of 14 microsatellites (Table 1). Our results corroborate with Coletta *et al*. (2010) and Albuquerque (2005) that estimated gene frequencies for the 13 microsatellite loci and concluded that excellent accuracy of PE (0.9999999% and 0.9998% respectively).

Other studies performed with *B. bubalis* have described some similar loci to our microsatellite polymorphism results: Muraleedharan *et al.* (2009) working on Indian water buffalo, Elbeltagy *et al*. (2008) working on Nile-Delta, Italian and Southern-Egypt buffalo, Zhang *et al*. (2007) working on Chinese indigenous buffalo and Albuquerque (2005) working on Brazilian buffaloes. Then, they reported number of alleles of 5, 7, 15, 12 vs. 5 (CSSM19). In additional authors, 8, 4, 10, 6 vs. 6 (CSSM38); 9, 9, 12, 16 vs. 13 (CSSM47) to Muraleedharan *et al*. (2009), Elbeltagy *et al*. (2008), Zhang *et al*. (2007) and Moioli *et al*. (2001) respectively. For this marker (CSSM47), Kumar *et al.* (2006) have found 9 alleles.

The primers used in the present study have been reported by different authors and also proved to be highly polymorphic in Brazilian Murrah breed: 6, 7 vs. 7 (CSSM42) by Elbeltagy *et al*. (2008) and Albuquerque (2005); 6, 11, 9 vs. 6 (CSSM60) by Elbeltagy *et al*. (2008), Zhang *et al*. (2007) and Moioli *et al*. (2001); 8, 7 vs. 9 (BM922) and 9, 9 vs. 8 (BM1706) by Muraleedharan *et al*. (2009) and Elbeltagy *et al*. (2008); 9, 10 vs. 6 (BMC1013) by Elbeltagy *et al*. (2008) and Moioli *et al*. (2001); 7 vs. 9 (INRA6); 10 vs. 7 (INRA26); 4 vs. 5 (CYP21); 6 vs. 4 (MAF65) and 9 vs. 6 (RM4) by Elbeltagy *et al*. (2008).

Different primers were analyzed and results were also efficient for example, 4, 6 and 8 (CSSM70) by Elbeltagy *et al*. (2008), Zhang *et al*. (2007) and Moioli *et al*. (2001); 11 and 9 (CA004) by Elbeltagy *et al*. (2008) and Kumar *et al.* (2006); 9 and 9 (BMS1747); 9 and 9 (BM757); 6 and 6 (BMS1724) by Muraleedharan *et al*. (2009) and Kumar *et al.* (2006), respectively.

Many other molecular markers have been tested in buffaloes and the results were polymorphic and highly heterozygosity primers as observed in this study (Muraleedharan *et al*., 2009; Nagarajan *et al*.,

Table 1. Genetic variability and probability of exclusion: number of alleles, Exclusion Probability of false parentage (PE), Polymorphic Information Content (PIC) and Heterozygosity value (Het.) in 100 Brazilian Murrah buffaloes

LOCUS	ALLELE	PE-1	PE-2	PE-3	PIC	Het.
BMC1013	6	0,304	0,476	0,653	0,674	0,723
BM1706	8	0,556	0,718	0,880	0,840	0,861
BM922	9	0,198	0,371	0,560	0,553	0,590
CSSM19	5	0,184	0,316	0,471	0,512	0,590
CSSM38	6	0,088	0,201	0,323	0,0361	0,416
CSSM42	7	0,141	0,305	0,488	0,474	0,502
CSSM47	13	0,308	0,492	0,697	0,667	0,697
CSSM60	6	0,164	0,331	0,514	0,509	0,543
CYP21	5	0,195	0,348	0,509	0,552	0,614
D5S2	1	0,000	0,000	0,000	0,000	0,000
INRA26	7	0,407	0,587	0,771	0,756	0,786
INRA6	9	0,508	0,678	0,850	0,819	0,839
MAF65	4	0,244	0,417	0,599	0,613	0,659
RM4	6	0,106	0,346	0,397	0,411	0,450
	92	**0,985424**	**0,999541**	**0,999998**		

PE-1: Parentage Exclusion Probability when only one parent is typed; PE-2: Parentage Exclusion Probability when both parents are typed; PE-3: Probability of Exclusion both parents; PIC: Polymorphism Information Content when both parents are typed; Het.: Heterozygosity value. Software Stormont Laboratories, Inc - Woodland - CA - USA, developed by Domenico Bernoco (UCDavis - CA - USA), in 1997 (under license).

2009; Zhang *et al*., 2007; Kumar *et al.*, 2006 and Navani *et al.*, 2002). An interesting result was the opportunity to verify the use of microsatellite markers in parentage DNA-test of the world.

CONCLUSIONS

The performance of this multiplex panel of markers suggests that it will be useful in parentage DNA test of Brazilian Murrah buffaloes, excepted for one marker (D5S2) which was monomorphic. By presenting only one allele, this marker should be excluded from the test and can be replaced by another marker. These results have similar specificity to those ones reported by Coletta *et al*. (2010).

This panel is already used in Italy for the Mediterranean buffaloes, one of the four breeds raised in Brazil. However, it is highly recommended that new loci are analyzed in order to increase the microsatellites panel repertoire used for genealogical studies.

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