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Evaluation of parentage testing in the Turkish Holstein population based on 12 microsatellite loci

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ABSTRACT

In the present study, 12 microsatellite loci (ETH10, ETH225, ETH03, TGLA122, TGLA227, BM1824, BM2113, INRA23, SPS115, TGLA126, RM006 and BM1818) were evaluated for their possible use to confirm selected pedigree relationships between 7 bulls, their 21 male offspring, and their 64 second-generation female offspring within the progeny test started in Turkey. The nine loci (BM1824, INRA23, BM2113, SPS115, ETH10, TGLA122, ETH225, TGLA126 and TGLA227) recommended by ISAG displayed high values for the measures of informativeness (allele numbers, heterozygosity, polymorphic information content, frequency of the most common allele, and power of discrimination). When both parents are known calculated combined probability of exclusion was at least 0.999. Range of probability of paternity (POP) values were 0.814–0.9999. Except 3 cases (4.7%), the alleged paternity relationships were confirmed. To have a higher confidence in POP values new loci must be integrated into the set of 9 loci used.

1. Introduction

To improve productivity in the animal industry selective breeding programs are utilized. In these programs progeny testing is employed to determine the value of the candidate animal to be used for breeding. For example, in dairy cattle to improve milk yield female offspring of a candidate bull are evaluated with respect to milk production, thereby estimating performance of the bull and possible use of its male offspring for reproduction in future generations is determined. Accurate pedigree information is of a paramount importance for a successful breeding program. Beginning in 1995 microsatellite loci was used to acquire pedigree information in livestock (Pepin et al., 1995; Ron et al., 1996; Bowling, 1997; Bowling, et al., 1997; Luikart et al., 1999).

The advantage of microsatellite based tests is several: any sample containing the animal's DNA, such as: hair, saliva, milk, vaginal swabs or semen can be used (Visscher et al., 2002; Baron et al., 2002; Ron et al., 2003) and these samples can be used retrospectively (Visscher et al., 2002). Accuracy of the test is high as microsatellite loci usually exhibit high number of alleles, and by polymerase chain reaction (PCR) concerted amplification of several loci (Heyen et al., 1997) and the genotyping of many individuals by automated processes enables direct and economical results (Řehout et al., 2006).

For parentage testing, initially 16 microsatellite loci were used by the Holstein Association USA, Inc. (1995 USA/CS-ISAG comparison test). Later, the International Society of Animal Genetics (ISAG) suggested a panel which included 9 of the previous 16 loci (BM1824, INRA23, BM2113, SPS115, ETH10, TGLA122, ETH225, TGLA126 and TGLA227) to be used in cattle parentage analysis (ISAG Conference, 2006).

These loci are highly informative (high number of alleles, high expected heterozygosity, high polymorphic information content, frequency of the most frequent allele: $p<0.5$, high power of discrimination and high probability of exclusion) as is seen in many studies (e.g., Bredbacka and Koskinen, 1999;

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Kovar et al., 2000; Radko et al., 2005; Řehout et al., 2006; Visscher et al., 2002; Curi and Lopes, 2002), therefore these loci are effective in parentage testing. Finally, probability of exclusion for all loci, i.e. combined probability of exclusion must be >0.999 for the acceptable level of the microsatellite loci in parentage testing (Vankan and Faddy, 1999; Chakraborty et al., 1988). To accept the parent as that of the offspring, the level of confidence must be such that probability of paternity (POP) is higher than 0.9973 (Vogt et al., 1991; Cerit, 2003).

In general, incorrect paternity information impairs a husbandry program (Baron et al., 2002; Visscher et al., 2002; Ron et al., 1996). Pedigree errors when using microsatellite loci have been reported in several studies (Ron et al., 1996; Visscher et al., 2002; Curi and Lopes, 2002; Ron et al., 2003; Weller et al., 2004; Řehout et al., 2006). For example, in two separate studies on Israeli Holstein, the proportion of misidentified progeny was 2.9% (Ron et al., 1996) —11.7% (Weller et al., 2004).

Usage of herd book rules and progeny testing were very rarely utilized in Turkey until recently. However, to advance husbandry techniques and select genetically superior bulls by using the production performance of its female offspring, Cattle Breeders Association of Turkey (CBAT), and Ministry of Agriculture and Rural Affairs have recently launched a wide scale progeny-testing program in Turkey. In the present study 12 microsatellite loci were used to confirm selected pedigree relationships within the progeny test started in Turkey. These loci were evaluated for their possible use in further studies. Furthermore, the misidentified progeny rate in relation to parentage testing was estimated in the Turkish Holstein population in Turkey.

2. Materials and methods

2.1. Population

Studied population was composed of 7 superior bulls, their male offspring, 21 candidate bulls, female offspring of 8 randomly selected candidate bulls (64) and their mates (64), representing a total of 156 individuals genotyped for this study. Female offspring and their mothers were born in different herds in 5 different provinces (Çanakkale, Kırklareli, Edirne, Balıkesir, Bursa), and the herds were raised by small enterprises. Populations investigated were sampled from members of the Cattle Breeders Organization of Turkey (CBAT) who participated in the progeny test project, consisting of the breed called Black and White (B&W) cattle (referred to as Turkish Holstein in the text), purebred or crossbred groups which originated in several countries including Holstein of Holland, USA, Germany, Canada, Italy and Israel.

2.2. Microsatellites analysis

All 12 microsatellites (ETH10, ETH225, ETH3, TGLA122, TGLA227, BM1824, BM2113, INRA23, SPS115, TGLA126, RM006 and BM1818) used in this study are recommended for bovine paternity tests by the Holstein Association USA, Inc. (1995 USA/CS-ISAG comparison test).

Genomic DNA was extracted from 10 ml blood samples using standard phenol-chloroform technique (Sambrook et al., 1989). All DNA extraction and PCR amplification were performed by the Middle East Technical University Department of Biology. The PCR analyses were carried out using a MyCyler Thermal Cycler (Bio-Rad). The reaction mixture was composed of genomic DNA (100 ng), 200 mM dNTPs, 2.0 mM MgCl2, 1X PCR buffer, 2.5 pmol forward and reverse primers and Taq DNA polymerase (0.5 u / sample) in a total volume of 20 µl. Microsatellite were amplified in a Bio-Rad Thermocycler using the following PCR program: 1 cycle of initial denaturation for 5 min at 94 °C, 30 cycles of 30 s at 94 °C, 90 s at annealing temperature, 1 min at 72 °C and 1 cycle of final extension for 10 min at 72 °C. Qiagen multiplex PCR kits were used to amplify multiple microsatellite loci. Amplified DNA was verified by electrophoresis of PCR mixtures in 2% agarose gel in 1X TBE buffer. After electrophoresis for all microsatellites, allele size was determined on all samples with an ABI Prism 310 Genetic Analyzer (Applied Biosystems) using the GeneScan Analysis Software (Applied Biosystems), which detects different alleles through size comparison with standard DNA size markers Tamra (Applied Biosystems).

2.3. Statistical analysis

The number of allele (n_A), observed (H_o) and expected heterozygosity (unbiased – H_e) (Nei, 1978), polymorphic information content (PIC) (Botstein et al., 1980), power of discrimination (PD) (Reis et al., 2008; Cerit, 2003), probability of exclusion (PE) (Curi and Lopes, 2002; Cerit, 2003; Řehout et al., 2006), and the paternity index (PI) were calculated for each microsatellite based on the parents' allele frequencies. All of these measures of informativeness were calculated using the Genetix (4.05) (Belkhir et al., 1996–2000), GenAlEx 6 (Peakall and Smouse, 2006), Cervus 3.0 (Marshall, T., 1998/2006) and PowerStatsV12 programs (Brenner and Morris, 1990a). n_A, H_o and H_e were calculated as given by Nei (1978, 1987), and PIC as was formulated by Botstein et al. (1980). PD was calculated as was by Kimberly (2001) and as defined by Brenner and Morris (1990b). Combined power of discrimination (CPD) for n loci was also calculated (Kimberly, 2001; Brenner and Morris, 1990b). PE was defined for three alternative cases (Jamieson, 1994; Jamieson and Taylor, 1997); PE_1 estimates the probability of exclusion of a parent when genotypes of the offspring and both its parents are known; PE_2 estimates the probability of exclusion of a parent when genotypes of the offspring and only one of its parents is known; and PE_3 estimates the probability of excluding two putative parents when genotypes of the offspring and both of its parents are known (Jamieson, 1994; Jamieson and Taylor, 1997). Combined probabilities of exclusion (CPE) over n unlinked loci in all three of the above cases were also calculated (Jamieson and Taylor, 1997). To calculate paternity index, the genotype frequencies of the putative parents were examined for each locus. For the 21 candidate bulls only the genotypes of fathers were available. Appropriate formulae given by Morris (1983) were used to calculate the paternity indices.

Combined paternity index was calculated in accordance with Morris' (1983) article, as it was referred by Brenner and Morris (1989) and Ostrowski (2006). Finally, probability of paternity (POP) was calculated as given by Morris (1983).