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APPLICATION OF A COMPLEMENTARY SET OF 10 MICROSATELLITE DNA MARKERS FOR PARENTAGE VERIFICATION IN POLISH RED CATTLE*

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Abstract

The objective of the study was to determine the polymorphism of 10 microsatellite DNA loci that are an additional set of DNA markers for cattle parentage verification at the National Research Institute of Animal Production and to assess its usefulness for Polish Red cattle. According to preliminary analysis, the estimated polymorphism of selected 10 markers shows that these markers could be useful in cases of disputed parentage in which the standard set may prove insufficient, for example in closely related animals or those subjected to rigorous selection.

Key words: Polish Red cattle, DNA microsatellites, polymorphism

At the end of the 1990s, cattle parentage tests based on blood groups were expanded to include DNA marker tests. Current routine tests use a set of 11 microsatellite DNA tests: TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, ETH3, ETH225 and BM1824, of which 9 (except TGLA53 and ETH3) are the minimum set recommended by the International Society for Animal Genetics (ISAG) for parentage verification.

The use of microsatellite DNA markers for animal parentage verification shows an over 99.9% probability that pedigree data are correct. In some cases, however, the use of a recommended marker panel may prove inadequate, for example when analysing closely related animals derived from twin pregnancies or from herds subjected to rigorous selection. In this connection, the Department of Animal Immuno- and Cytogenetics of the National Research Institute of Animal Production has chosen a set of 10 microsatellite DNA sequences as a complementary panel of DNA markers. These markers (CSRM60, ILSTS065, CSSM066, BM1818, INRA072,

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BM2830, AGLA293, INRA222, INRA092 and HUJI177) are used also by other international laboratories for parentage verification in cattle.

The objective of the study was to determine the polymorphism of selected markers and their usefulness for parentage verification in Polish Red cattle.

Material and methods

Blood samples from cattle subjected to routine parentage testing at the National Research Institute of Animal Production were used to identify the polymorphism of selected microsatellite DNA sequences. A total of 113 Polish Red (PR) cattle were investigated.

Ten microsatellite DNA sequences, reported in certificates issued by other laboratories for bulls subjected to routine parentage tests, were analysed. The following panel of microsatellite sequences was tested in the Institute: CSRM60, ILSTS065, CSSM066, BM1818, INRA072, BM2830, AGLA293, INRA222, INRA092 and HUJI177.

Genomic DNA was isolated from blood samples and hair bulbs using proteinase K according to the method described by Kawasaki (1990) and from semen using a Promega Wizard kit for isolation from tissues according to the manufacturer's protocol.

Isolated DNA was used to amplify selected DNA fragments by multiplex PCR, using fluorescently labelled primer sequences and TaqGold polymerase. Amplification was performed using a GeneAmp PCR System 9600 (Applied Biosystems) and the following thermal programme: initial DNA denaturation, 3 min at 98°C, followed by 30 cycles of denaturation at 98°C for 15 s, annealing at 57°C for 75 s, elongation at 72°C for 30 s and a final elongation step at 72°C for 60 min.

The PCR products obtained were electrophoresed in a 7% denaturing polyacrylamide gel using the 350 Rox standard in a 3100xL sequencer. The results of electrophoretic separation (DNA fragments of different lengths) were read using GeneMapper software.

The data obtained were used for statistical analysis. Estimations were made of heterozygosity (H) (Ott, 1992), polymorphic information content (PIC) (Botstein et al., 1980), parentage exclusion probability (PE) for each locus when the genotypes of both parents are known (Jamieson, 1965) and combined probability of parentage exclusion (PEc) for all 10 loci together (Fredholm and Wintero, 1996).

Calculations were performed using Excel 97, Px and Tfpga 2000 version 1.3 (Tools for Population Genetic Analyses) software.

Results

The PCR products obtained in all analysed loci made it possible to determine alleles for different markers (Figure 1).

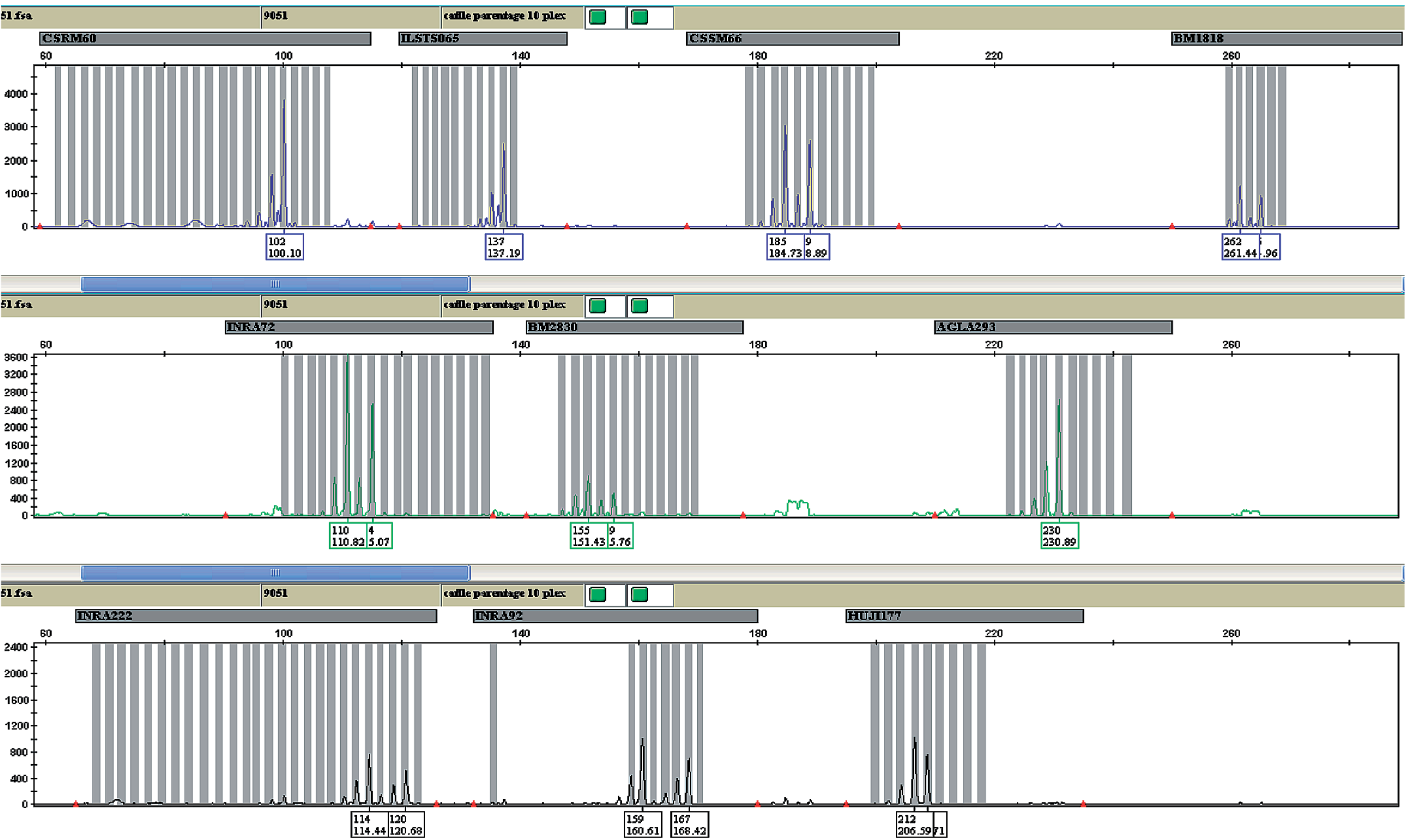

Figure 1. Results of electrophoretic separation of 10 microsatellite DNA sequences on a 3100xL sequencer

In the analysed cattle, 78 alleles whose number ranged from 5 (locus INRA072) to 13 (locus BM2830) depending on locus, were detected in 10 microsatellite DNA loci (Table 1).

Polymorphic information content (PIC) and heterozygosity (H) were estimated for different loci based on calculated allele frequencies. All the loci under analysis show high PIC and H values (>0.6) except locus AGLA293, for which the lowest values of 0.445 and 0.463, respectively were noted. The highest values of these parameters (>0.8) were determined for the BM2830 and INRA222 loci (Table 1). The probability of parentage exclusion (PE) in the cattle breed studied, calculated based on single loci, ranged from 0.287 (AGLA293 locus) to 0.701 (BM2830 locus). This parameter had high values (>0.5) in 7 loci and low values in only 3 loci – AGLA293, BM1818 and INRA222 (Table 1). Combined probability of parentage exclusion (PE_C) calculated from 10 loci was 0.9997.

Table 1. Microsatellite DNA markers and their usefulness for Polish Red cattle studied

	No. of alleles	Allele size range	H	PIC	PE
CSRM60	6	92–104	0.782	0.748	0.573
ILSTS065	7	127–143	0.756	0.724	0.550
CSSM066	10	179–199	0.791	0.768	0.612
BM1818	6	258–268	0.674	0.621	0.422
INRA072	5	110–124	0.683	0.631	0.434
BM2830	13	151–179	0.845	0.829	0.701
AGLA293	7	222–240	0.463	0.445	0.287
INRA222	10	96–122	0.847	0.829	0.696
INRA092	8	133–169	0.795	0.765	0.601
HUJI177	6	206–212	0.767	0.729	0.546

H – degree of heterozygosity
PIC – polymorphic information content
PE – probability of exclusion

Discussion

At present, parentage verification at the National Research Institute of Animal Prodcution is carried out based on a recommended panel of 11 DNA markers (Radko, 2008). The number of cattle analysed to verify pedigree data was over 1000 in 2006, about 3000 in 2007 and over 5600 in 2008. In 2009 it is planned to test over 7000 head of cattle. Considering such a large number of tests, there is a risk that in some cases (e.g. animals from herds subjected to rigorous selection or closely related animals), the panel of 11 DNA markers may prove insufficient to conclusively verify pedigree data. Where a mismatch in genotype between offspring and its potential parents occurs for only one locus, and assuming that a mismatch at one locus does not indicate exclusion, the use of additional markers takes on significance (Weller et al., 2004). Determination of genotypes at additional loci will confirm or exclude

parentage of the animal tested. Out of almost 1200 expert analyses performed to date that have confirmed parentage, additional markers had to be used in 29 cases to determine whether differences in the profile are caused by laboratory or pedigree.

Many laboratories use other microsatellite sequences in addition to the basic set of 9 microsatellites. DNA certificates provide additional genotypes at the following loci: HUJI177, ILSTS65, INRA72, INRA92, INRA135, INRA177 and INRA222 (Labogena laboratory, France); BM1818, CYP21, RM67, MGTG4B and SPS113 (Holstein Association laboratory, USA); and BM1818, CSSM066 and HEL1 (Canada).

No agreement has been reached yet concerning the additional, complementary panel of markers that could be used, if necessary, in international sets and in cases of disputed parentage. For this reason, the Department of Animal Immuno- and Cytogenetics of the National Research Institute of Animal Production has determined an additional set of microsatellite loci, which were chosen based on the list of markers specified in certificates issued by other laboratories, were tested in international comparison tests (ISAG) and had specific (internationally standardized) alleles, making it possible to compare results between laboratories. Selected markers were amplified in a single multiplex PCR, which shortens the time of analysis and makes it less expensive.

In the present study, PCR products obtained from different loci were of good quality and enabled alleles to be determined for all markers studied (Figure 1).

Preliminary analysis was performed on 113 head of Polish Red cattle. In the population studied, the frequency of alleles detected at 10 microsatellite DNA loci varied according to locus. The highest frequency (>0.7212) was found for one allele of 230 bp at the AGLA293 locus. High frequencies of 0.469 and 0.443 were found for a 266 bp allele at the BM1818 locus and a 114 bp allele at the INRA072 locus, respectively. The other alleles had a frequency below 0.4. The lowest frequency (<0.009) was characteristic of 3 alleles (151 bp, 234 bp and 258 bp) at the BM2830, AGLA293 and BM1818 loci, respectively.

PIC and H values, calculated from allele frequency, showed high polymorphism in 9 loci analysed in the PR breed of cattle (Table 1). The highest PIC and H values of over 0.84 were found for the BM2830 and INRA222 loci, and in the other loci they exceeded 0.6 except the AGLA293 marker, which showed the lowest polymorphism. Out of 7 alleles determined at this locus, one had a frequency of 72%, with PIC and H values of 0.445 and 0.463, respectively.

The probability of parentage exclusion (PE) directly indicates the usefulness of microsatellite markers for parentage verification. PE can be calculated based on a single locus or all loci (PE_C).

The combined probability of exclusion, estimated from different sets of microsatellite markers, usually exceeds 99% (Usha et al., 1995; Bates et al., 1996; Holm and Bendixen, 1996; Heyen et al., 1997; Baron et al., 2002; Radko et al., 2002; Cervini et al., 2006). Based on the set of 11 markers recommended by ISAG for parentage verification, PEC calculated for Polish Red cattle was 99.9% (Radko, 2008).

In the PR cattle population investigated, PE ranged from 0.287 (AGLA293 locus) to 0.701 (BM2830 locus) according to marker. The high values of this param-

eter were found for CSSM066, INRA222 and INRA092 (PE>0.6) and CSRM60, ILSTS065 and HUJI177 (PE>0.5) (Table 1). The combined probability of parentage exclusion, estimated based on the additionally analysed 10 loci, was 0.9997. It can be expected that PEc calculated concurrently based on the basic panel of 9 markers used for parentage verification and the complementary set of 10 markers will increase the probability of correct pedigree data to values near 100%.

In conclusion, the polymorphism of 10 analysed markers shows that the complementary set of microsatellite DNA sequences could be used for confirming parentage in cattle. This particularly refers to imported animals, which in addition to genotype determined in the loci of markers from the basic parentage verification panel have genotype determined in the loci of additionally tested markers. The amount of additional analyses (about 0.8%) demonstrates that this set will find application in the laboratory of the Department of Animal Immuno- and Cytogenetics of the National Research Institute of Animal Production only when necessary, for example in cases of disputed parentage when the standard panel is inadequate.

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Zastosowanie uzupełniającego zestawu 10 markerów mikrosatelitarnych DNA do weryfikacji rodowodów bydła polskiego czerwonego

STRESZCZENIE

Celem przedstawionej pracy było określenie polimorfizmu 10 loci mikrosatelitarnych DNA, stanowiących uzupełniający zestaw markerów DNA do weryfikacji pochodzenia bydła w Instytucie Zootechniki PIB oraz ocena jego przydatności u bydła rasy polskiej czerwonej. We wstępnej analizie wykazano, że oszacowany polimorfizm wybranych 10 markerów wskazuje, że markery te mogą być przydatne w rozstrzyganiu spraw dotyczących spornego pochodzenia, gdzie standardowy zestaw może okazać się niewystarczający, na przykład u zwierząt blisko spokrewnionych lub pochodzących ze stad podlegających ostrej selekcji.