Ann. Anim. Sci., Vol. 9, No. 2 (2009) 119–125

USE OF BLOOD GROUP TESTS AND MICROSATELLITE DNA MARKERS FOR PARENTAGE VERIFICATION IN A POPULATION OF POLISH RED-AND-WHITE CATTLE

Anna Radko, Tadeusz Rychlik

Department of Animal Immuno- and Cytogenetics, National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland

Abstract

The polymorphism of erythrocyte antigens in 12 blood group systems was tested in 744 Red-and-White cattle from south and south-west Poland. Microsatellite DNA polymorphism was analysed using 128 blood samples taken from animals randomly selected from this group. The polymorphism of the markers studied was determined based on 149 alleles identified in 12 blood group systems and 79 alleles in 11 microsatellite sequences. The highest polymorphism was found in the A, B, C and S blood group systems, in which H and PIC values ranged from 0.655 and 0.591 in the S system to 0.947 and 0.929 in the B system. In the F, J, L, M, Z, N', R' and T' systems, H and PIC values were less than 0.5. Growing demand for high quality animal products and the search for quicker and more reliable methods of livestock identification have increased the scope of recent animal genetic variability studies with DNA marker analysis. Microsatellite sequences, 1–5 nucleotide sequences, and DNA tandem repeats, also known as STR (short tandem repeats) have found the widest application. The analysed microsatellite markers (TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, ETH3, ETH225, BM1824) were characterized by high polymorphism of the material studied. Polymorphic information content (PIC) estimates exceeded 0.7 for the TGLA227, BM2113, TGLA53, TGLA122, INRA23 and ETH225 loci. For the other loci, PIC exceeded 0.5 and H ranged from 0.597 (SPS115) to as much as 0.863 (TGLA53). The highest polymorphism was characteristic of the TGLA53 marker, for which PIC was 0.849 and H was 0.863. Based on the estimated probability of exclusion (PE_C), it was found that incorrect parentage assignment can be excluded with 99.58% probability using blood group systems and with as much as 99.987% probability using microsatellite DNA analysis.

Key words: cattle, erythrocyte antigens, DNA microsatellites, parentage control

The cattle parentage verification and identification system based on blood group tests has been used in Poland since the 1960s. Cattle pedigree data are confirmed based on erythrocyte antigens of 12 blood groups, which are determined using over 70 test sera. The probability of parentage exclusion based on the polymorphism of

erythrocyte antigens exceeds 98% (Holm and Bendixen, 1996). In herds of cattle with an elevated inbreeding coefficient, homozygosity is increased and the gene pool limited. In this situation, paternity testing based on serological tests is often difficult or even impossible. In cases like these, microsatellite DNA sequences are increasingly used for pedigree verification in addition to blood groups (Heyen et al., 1997; Peelman et al., 1998; Radko et al., 2002). The current set of 11 highly polymorphic microsatellite markers estimates the probability of incorrect parentage assignment with 99.9% accuracy (Radko, 2008).

The aim of the study was to compare the usefulness of blood group tests and microsatellite DNA sequences for parentage verification in a population of Polish Red-and-White cattle.

Material and methods

The polymorphism of erythrocyte antigens in 12 blood group systems was tested in 744 Red-and-White cattle from south and south-west Poland. Microsatellite DNA polymorphism was analysed using 128 blood samples taken from animals randomly selected from this group.

Erythrocyte antigens in the A, B, C, F, J, L, M, S, Z, N', R' and T' systems were identified using 78 test reagents in the hemolytic test. All of the test reagents, obtained at the Department of Animal Immuno- and Cytogenetics of the National Research Institute of Animal Production, were standardized in international comparison tests organized by the International Society for Animal Genetics (ISAG).

DNA polymorphism was analysed using 11 microsatellite loci recommended by ISAG for parentage verification in cattle: TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, ETH3, ETH225 and BM1824. These markers are part of the StockMarks for Cattle II kit (Applied Biosystems).

Based on the genomic DNA isolated, the amplification of sequences from selected loci was performed by polymerase chain reaction (PCR) using fluorescently labelled primers. The markers were amplified in a single 11-plex reaction mixture. The amplification conditions followed the Protocol for Bovine II Version 2 (Mixed 11 Plex Kit) by Applied Biosystems.

The PCR products obtained were analysed using an ABI 3130xl capillary sequencer (Applied Biosystems). Amplified DNA fragments of different length were electrophoresed in 7% denaturing polyacrylamide gel POP-7, in the presence of a GS500-ROX size standard and reference sample. The results of electrophoretic separation were analysed automatically using GeneMapper software.

The frequency of individual blood group alleles and microsatellite sequences was used to calculate the degree of heterozygosity (H) and polymorphic information content (PIC). The probability of paternity exclusion (PE), taking into account the possibility of testing both parents, was calculated for each locus and separately for blood groups and microsatellite DNA markers.

Results

A total of 149 alleles in 12 blood group systems were identified in the present study. The highest number of alleles (72) was determined in the B blood group system and the lowest (2) in the F, J, L, M, Z, N', R' and T' systems.

A total of 79 alleles were identified in microsatellite DNA markers. The highest number of alleles (11) was identified at the TGLA53 locus, 10 alleles were observed at each of the TGLA227 and TGLA122 loci, and the smallest number of alleles (5) was obtained at the SPS115, TGLA126 and BM1824 loci (Table 1).

Table 1. Number of identified alleles (n), coefficient of heterozygosity (H), polymorphic information content (PIC) and probability of exclusion (PE) for the analysed markers in Red-and-White cattle

	Marker	n	PIC	H	PE
Blood groups	EAA	11	0.671	0.714	0.483
	EAB	72	0.929	0.947	0.864
	EAC	44	0.878	0.881	0.781
	EAF	2	0.171	0.190	0.086
	EAJ	2	0. 255	0.297	0.128
	EAL	2	0.308	0. 379	0.154
	EAM	2	0. 033	0.034	0.016
	EAS	6	0. 591	0.655	0.392
	EAZ	2	0. 299	0.364	0.149
	EAN'	2	0. 276	0.322	0.138
	EAR'	2	0.060	0.061	0.030
	EAT'	2	0. 128	0.137	0.064
	$\bar{x}$		**0. 375**	**0.416**	**PE_{12loci} = 0.99590**
Microsatellite DNA markers	TGLA227	10	0.780	0.802	0.628
	BM2113	6	0.720	0.757	0.538
	TGLA53	11	0.849	0.863	0.729
	ETH10	7	0.581	0.612	0.402
	SPS115	5	0.546	0.597	0.355
	TGLA126	5	0.592	0.657	0.389
	TGLA122	10	0.781	0.804	0.629
	INRA23	7	0.792	0.818	0.637
	ETH3	6	0.680	0.719	0.496
	ETH225	7	0.731	0.768	0.550
	BM1824	5	0.654	0.699	0.462
	$\bar{x}$		**0.701**	**0.736**	**$PE_{111loci}$ = 0.99987**

Based on the frequency of identified alleles, the polymorphic information content and the degree of heterozygosity were determined.

For blood groups, PIC values ranged from 0.033 (EAM system) to 0.929 (EAB), and H values from 0.034 (EAM) to 0.947 (EAB).

Within microsatellite markers PIC values ranged from 0.546 (SPS115 locus) to 0.849 (TGLA53), with H values ranging from 0.597 (SPS115) to 0.863 (TGLA53) (Table 1).

The probability of parentage exclusion, calculated in the group systems, ranged from 0.016 for EAM to 0.864 for EAB. For microsatellite markers, PE was the highest (0.729) for the TGLA53 locus and the lowest (0.355) for SPS115. For the other loci, PE values ranged from 0.389 (TGLA126 locus) to 0.637 (INRA23 locus) (Table 1). The combined probability of exclusion (PE_C), estimated for the analysed cattle was 0.9959 based on blood groups and 0.99987 for 11 DNA loci.

Discussion

The use of techniques based on DNA analysis in parentage verification made it possible to develop rapid and accurate methods for identification of breeding animals. Many studies have shown the high usefulness of microsatellite DNA sequences for parentage verification in different species of breeding animals such as horses, cattle and sheep (Janik et al., 2003; Rychlik et al., 2003; Ząbek et al., 2003). In Poland, cattle parentage verification based on blood group tests has been extended with DNA markers since 2007. Today, the polymorphism of microsatellite DNA loci is analysed at the Department of Animal Immuno- and Cytogenetics of the National Research Institute of Animal Production in routine cattle parentage testing.

A total of 149 alleles in 12 blood group systems were identified in the present study.

The highest number of alleles was determined in the B and C blood group systems (72 and 44, respectively), with 11 alleles found in the A system, 6 in the S system and 2 alleles in the F, J, L, M, Z, N', R' and T' systems. Within microsatellite DNA markers, 79 alleles were identified, with 5 to 11 alleles per locus (Table 1).

Based on the alleles identified, the material studied was analysed for the polymorphism of genetic markers used for parentage verification in cattle, by determining the polymorphic information content (PIC) and the degree of heterozygosity (H).

The considerable differences in the allele pool between individual blood group systems had little effect on the level of polymorphism in the analysed loci of erythrocyte antigens. The mean PIC and H values for blood groups were 0.375 and 0.416, respectively.

The highest polymorphism was observed in the B, C, A and S blood group systems, where PIC and H ranged from 0.591 and 0.655 (EAS) to 0.929 and 0.947 (EAB), respectively. In the F, J, L, Z, N' and T' systems, PIC and H ranged from 0.128 and 0.139 (EAT') to 0.299 and 0.364 (EAZ), respectively. In the EAM and EAR' systems, in which one of the two alleles identified occurred with much greater frequency, PIC and H values were low at 0.033 and 0.034 for EAM and at 0.060 and 0.061 for EAR,

respectively. This indicates that these markers are not very useful in parentage verification of the cattle studied.

Studies of the genetic structure of Red-and-White cattle in 2001–2006 (Rychlik et al., 2008) showed that genetic variation decreased in relation to earlier research (Trela, 1977). The number of alleles in the B blood group system, which is used the most for parentage verification, decreased considerably from 143 to 72, which makes it more difficult to determine parentage based on blood groups. Also studies by different authors who described the genetic structure of other cattle breeds based on blood group tests showed that these markers became less varied (Walawski, 1976; Rychlik et al., 1999; Duniec et al., 2002).

Due to their high degree of polymorphism, microsatellite DNA markers have been successfully used for many years to verify parentage of farm animals and to determine genetic variation within individual populations (Radko et al., 2004; Cervini et al., 2006; Rehout et al., 2006).

The genetic variation among Polish Red-and-White cattle based on microsatellite markers was already analysed in 1998–2001 (Radko et al., 2002) and revealed a high degree of polymorphism of the markers investigated. In this study, PIC and H values exceeded 0.8 for the TGLA227, TGLA122, INRA23 and ETH225 loci. The highest polymorphism was characteristic of the TGLA227 locus (PIC = 0.844, H = 0.859). For the other loci, these parameters exceeded 0.7 except the SPS115 locus, for which PIC was 0.613 and H was 0.654.

The mean values of PIC and H parameters were 0.761 and 0.788, respectively.

The present analysis of genetic variation in Red-and-White cattle also showed that the polymorphism of the analysed DNA sequences was considerable but perceptibly lower than that found in earlier research.

PIC and H values in excess of 0.8 were only observed for the TGLA53 marker (PIC = 0.849, H = 0.863), which showed the highest polymorphism in the population studied. PIC and H values exceeding 0.7 were noted at the TGLA227, BM2113, TGLA122, INRA23 and ETH225 loci. The lowest polymorphism was found at the SPS115 locus (PIC = 0.546, H = 0.597).

Mean PIC and H values of 0.701 and 0.736 were lower than previous ones by 7.8% and 6.6%, respectively.

Another parameter that directly determines the usefulness of polymorphic genetic markers for parentage verification is the probability of incorrect parentage assignment (PE).

Studies by other research centres showed that PEC was 0.99 when calculated based on just 6 microsatellite sequences (CSM42, BM2113, ETH225, INRA23, BM1824 and ETH3) and 0.98 when calculated using the analysis of 11 blood group systems (Holm and Bendixen, 1996).

In our study, the probability of exclusion was estimated separately for each locus studied while accounting for the possibility of testing both parents, as shown in Table 1.

Based on the PE values obtained for different markers, the combined probability of exclusion (PE_C) was estimated for polymorphic blood groups and 11 microsatellite markers. It was found based on PE_C that incorrect parentage assignment can

be excluded with 99.58% probability using blood group systems and with as much as 99.987% probability using DNA analysis. The high probability of exclusion ($PE_C = 0.999$), calculated based on microsatellite DNA markers recommended by the ISAG for parentage verification, was also found in other cattle breeds (Bredbacka and Koskinen, 1998; Radko et al., 2002; Janik et al., 2003).

The evaluation of genetic markers used to verify cattle parentage demonstrated that in closed and relatively small populations and in herds subjected to rigorous selection, genetic variation may be limited, as reflected in the polymorphism of genetic markers and their usefulness for parentage tests.

The polymorphism of the DNA markers, recommended by the ISAG for cattle parentage and blood group verification and determined based on the estimated PIC, H and PE values, shows that they are useful for parentage verification of the cattle studied. However, the downward tendency of genetic variation suggests that breeders should pay more attention to preservation of biodiversity among the Polish Red-and-White breed of cattle.

The current parentage verification of cattle in Poland is conducted based on blood groups and DNA polymorphism. DNA tests cover mainly breeding bulls and heifers, whereas the polymorphism of erythrocyte antigens is studied mainly in beef cattle. Although blood group and DNA marker studies are treated on an equal basis, the results of DNA tests are increasingly attached to certificates of origin in the European Union countries, in the USA and Canada. It should be expected that in the near future it will be obligatory to use DNA markers for parentage verification in cattle. The most important arguments for the use of this method is the possibility of using different biological materials for research, the accurate and rapid method of their analysis through the use of automated DNA sizing technology in sequencers, and almost 100% probability of exclusion.

References

Bredbacka P., Koskinen M.T. (1998). Polymorphism of microsatellite loci used in parentage testing in Finnish Ayrshire and Holstein populations. XXVIth Internat. Conf. Animal Genetics; 9–14.08.1998, Auckland, New Zealand, p. 15.

Cervini M., Henrique-Silva F., Mortari N., Matheucci E. (2006). Genetic variability of 10 microsatellite markers in the characterization of Brazilian Nellore cattle (*Bos indicus*). Genet. Mol. Biol., 29: 486–490.

Duniec M., Rychlik T., Duniec M.J., Kościelny M. (2002). Blood group polymorphism in Simmental cattle raised in Poland. Ann. Anim. Sci., 2, 1: 41–51.

Heyen D.W., Beever J.E., Da Y., Evert R.E., Green C., Bates S.R.E., Ziegle J.S., Lewin H.A. (1997). Exclusion probabilities of 22 bovine microsatellite markers in fluorescent multiplexes for semiautomated parentage testing. Anim. Genet., 28: 21–27.

Holm L-E., Bendixen C. (1996). Usefulness of microsatellites from the ISAG comparison test for parentage control in Danish Black-and-White cattle. Anim. Genet., 27: 17–42.

Janik A., Ząbek T., Radko A. (2003). Analysis of usefulness of 11 DNA microsatellites for parentage control in Limousin cattle. Ann. Anim. Sci., 3: 11–20.

Peelman L.J., Martiaux F., Van Zeveren A., Dansercoer A., Mommens G., Coopman F., Bouquet Y., Burny A., Renaville R., Portetelle D. (1998). Evaluation of the genetic variability of 23 bovine microsatellite markers in four Belgian cattle breeds. Anim. Genet., 29: 161–167.

Radko A. (2008). Microsatellite DNA polymorphism and its usefulness for pedigree verification of cattle raised in Poland. Ann. Anim. Sci., 8, 4: 205–216.
Radko A., Duniec M., Ząbek T., Janik A., Natonek M. (2002). Polimorfizm 11 sekwencji mikrosatellitarnych DNA i ocena ich przydatności do kontroli pochodzenia bydła. Med. Wet., 58 (9): 708–710.
Radko A., Żyga A., Słota E., Kościelny M., Brejta W. (2004). Ocena efektywności grup krwi i sekwencji mikrosatelitarnych DNA w kontroli rodowodów bydła. Med. Wet., 60 (11): 1212–1214.
Rehout V., Hradecká E., Cítek J. (2006). Evaluation of parentage testing in the Czech population of Holstein cattle. Czech J. Anim. Sci., 51, (12): 503–509.
Rychlik T., Duniec M.J., Duniec M., Kościelny M. (1999). Characteristics of the genetic structure of Polish Red cattle based on blood group studies. Ann. Anim. Sci., 4: 11–22.
Rychlik T., Radko A., Duniec M. (2003). Ocena przydatności polimorfizmu niektórych markerów genetycznych w kontroli rodowodów owiec. Med. Wet., 59: 1016–1018.
Rychlik T., Radko A., Kościelny M. (2008). Characteristic of the genetic structure of Red-and-White cattle based on blood group tests. Ann. Anim. Sci., 3: 215–223.
Trela E. (1977). Immunogenetyczna charakterystyka bydła rasy czerwono-białej na podstawie grup krwi i typów beta-globulin (transferryna). Rocz. Nauk. Zoot. Monogr. Rozpr.. 7: 49–77.
Walawski K. (1976). Zastosowanie grup krwi jako markerów zmian genetycznych spowodowanych selekcją bydła rasy nizinnej czarno-białej. Zesz. Nauk. AR-T Olsztyn, 10: 93–107.
Ząbek T., Duniec M., Słota E., Radko A. (2003). Markery mikrosatelitarne DNA w kontroli rodowodów u koni śląskich i pełnej krwi angielskiej. Med. Wet., 59: 529–531.

Accepted for printing 30 IV 2009

ANNA RADKO, TADEUSZ RYCHLIK

Wykorzystanie grup krwi i markerów mikrosatelitarnych DNA do weryfikacji rodowodów w populacji polskiego bydła czerwono-białego

STRESZCZENIE

Badania polimorfizmu antygenów erytrocytarnych w 12 układach grupowych krwi przeprowadzono u 744 sztuk bydła rasy czerwono-białej pochodzącego z obszaru południowej i południowo-zachodniej Polski. Analizę polimorfizmu mikrosatelitarnego DNA wykonano na 128 próbkach krwi od osobników losowo wybranych z tej grupy. Na podstawie 149 alleli zidentyfikowanych w 12 układach grupowych krwi i 79 alleli 11 sekwencji mikrosatelitarnych określono polimorfizm badanych markerów. Najwyższy polimorfizm zaobserwowano w układach grupowych krwi A, B, C i S, gdzie wyliczone parametry H i PIC wynosiły odpowiednio od 0,655 i 0,591 (dla układu S) do 0,947 i 0,929 (dla układu B). W układach grupowych krwi F, J, L, M, Z, N', R', T' wyliczone wartości H i PIC wynosiły poniżej 0,5.

Analizowane markery mikrosatelitarne: TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, ETH3, ETH225, BM1824 charakteryzowały się wysokim polimorfizmem w badanym materiale. Oszacowane wartości indeksu stopnia polimorfizmu – PIC osiągnęły ponad 0,7 dla loci TGLA227, BM2113, TGLA53, TGLA122, INRA23, i ETH225. Dla pozostałych loci wartości PIC wyniosły ponad 0,5, natomiast wartości H mieściły się w granicach od 0,597 (SPS115) aż do 0,863 (TGLA53). Najwyższym polimorfizmem odznacza się marker TGLA53, dla którego wartość PIC osiągnęła poziom 0,849 oraz H – 0,863. Na podstawie oszacowanego prawdopodobieństwa wykluczenia (PE_C) stwierdzono, że błędnie wpisanego rodzica można wykluczyć z 99,58% prawdopodobieństwem wykorzystując układy grupowe krwi, natomiast aż z 99,987% prawdopodobieństwem, przeprowadzając analizę mikrosatelitów DNA.