ANALYSES OF THE GENETIC DIVERSITY WITHIN LITHUANIAN WHITE-BACKED CATTLE

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Summary. The objective of this study was to use red cell antigen system to assess temporal changes in intrabreed genetic variation of Lithuanian White-Backed cattle under restoration. Now, native cattle with a white dorsal stripe and black or brown pigment side colour are an indigenous breed found mostly in the southeastern regions of Lithuania.

The blood samples of Lithuanian White-Backed cattle were classified in to five different groups. The animals from each group were not related, except the animals from groups 1, 2 and 4. The genetic markers used to characterize the intrabreed were 29 alleles in EAB and EAC systems of blood groups. Within-group variation was estimated by expected heterozygosity, allele frequencies and genetic identity (r).

The result is important since it shows that cattle from different herds have retained reasonably not high genetic diversity at the biochemical loci. Allele frequencies differentiation between the groups showed that all groups of LWB cattle were significantly different from one another. Genetic divergence of the LWB cattle in addition to within population genetic diversity is a result of the combined effects of breeding, geographic origin, the extent of admixture occurring during breed foundation and development.

The results suggest that there has been different gene flow among modern-day pedigree breeds of cattle in to the single animal from different regions, and it has resulted in significant genetic differentiation of the groups. Heterozygosities of LWB cattle were high and ranged between 0.793 and 0.923. Decrease of heterozygosities in progeny of conservation herd showed that consequently, to purifying the LWB population the breed could have lost 0.4% of their heterozygosity over few generations. The influence of selected bulls on animals from the conservation herd was defined sufficiently high (r=0.58 and 0.50).

Keywords: blood groups, White-Backed cattle, genetic variation, heterozygosity.

GENETINĖS ĮVAIROVĖS ANALIZĖ LIETUVOS BALTNUGARIŲ GALVIJŲ POPULIACIJOJE

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Santrauka. Šio darbo tikslas – pagal kraujo grupių sistemas nustatyti atkuriamos Lietuvos baltnugarių galvijų veislės laikinus pokyčius populiacijos viduje. Vietiniai galvijai su baltu dryžiu per nugarą ir juodo arba rudo pigmento šonais yra senovinė veislė, šiuo metu aptinkama daugiausia pietrytiniuose Lietuvos regionuose.

Tyrimui naudoti Lietuvos baltnugariai galvijai suskirstyti į penkias skirtingas grupes. Galvijai grupėse buvo negimininginiai, išskyrus I, II ir IV grupes. Galvijams charakterizuoti veislės viduje buvo naudojami 29 aleliai iš EAB ir EAC kraujo grupių sistemų. Įvairavimas tarp grupių buvo įvertintas nustatant heterozigotiškumą, alelių dažnį ir genetinį identiškumą (r).

Tyrimo rezultatai parodė, kad galvijai iš skirtingų bandų biocheminiuose lokusuose yra siauros genetinės įvairovės. Alelių dažnių skirtingumas tarp grupių parodė, kad Lietuvos baltnugariai galvijai patikimai skyrėsi vieni nuo kitų. Šių galvijų genetinis skirtingumas populiacijos viduje yra nesistemingo veisimo ir kilmės efekto rezultatas.

Tyrimo rezultatai parodo, kad šių laikų kilmės galvijų veislių genų įtaka atskirų regionų gyvuliams turėjo būti skirtinga, dėl to tarp grupių gauti patikimi skirtumai. Lietuvos baltnugarių galvijų heterozigotiškumas yra aukštas ir įvairavo nuo 0,7929 iki 0,9230. Sumažėjęs palikuonių heterozigotiškumas parodė, kad, gryninant Lietuvos baltnugarių galvijų populiaciją, per keletą kartų veislė gali prarasti 0,4 proc. heterozigotiškumo. Atrinktų veisimui bulių įtaka palikuonims ganėtinai didelė (r=0,50).

Raktažodžiai: kraujo grupės, baltnugariai, genetinė įvairovė, heterozigotiškumas.

Introduction. In 19th century, as regards colour diversity, four types of native cattle were found (Petraitis, 1955). Lithuanian White-Backed cattle were one-in-four also bred in Lithuania. In 20th century Lithuanian Red and Black-and-White cattle populations were bred in Lithuania (Vitkus, 1928). These breeds assimilated all native cattle branches with the exception of the White-Backed breed (Kuosa, 1997). The Holstein breed, which is composed almost completely of American Holstein genes, has largely replaced other breeds of dairy cattle throughout much of the world (Lush, 1945). Lithuanian cattle have also been subject to more recent introgression of Holstein. Therefore, native cattle breeds which have adapted to local environments during several generations of selection

are currently endangered. At present, native cattle with a white dorsal stripe and black or brown pigment side colour are an indigenous breed found mostly in the south-eastern regions of Lithuania. It is known that cattle similar to our white-backed cattle were also bred in the north-eastern regions of Poland, Scandinavian countries and some regions of Russia (Litwinczuk, 2002; Sæther, Vangen, 2001).

The restoration of Lithuanian independence enabled to change the views of the society regarding the conservation of the native cattle breeds. Since 1994, conservation started by finding the most typical animals on private farms of Lithuania (Šveistys, 1997; 1998). It enabled to restitute the population of LWB cattle, and Ministry of Agriculture acknowledged it the Lithuanian pedigree, what resulted in the breeding registers opening. Three indigenous cattle herds were formed by pure breeding. In LWB cattle pedigree information is owned and managed by milk recording organization, however in other countries (and some Lithuanian cattle breeds) this information is often recorded, owned and managed by breed societies.

In cattle, analysis of allelic variation at red cell antigen loci could potentially be used to evaluate temporal changes in genetic diversity. Previous studies on genetic diversity in cattle are based on data derived from typing of red cell antigens and plasma proteins. These genetic systems have been studied in Lithuanian cattle for several decades starting from the study by Meškauskas (1967) whereas separately Lithuanian White-Backed cattle were analyzed by J. Kuosa and Tušas (1999).

Recently, increased preference has been given to microsatellites that are ubiquitous throughout the cattle genome and highly polymorphic. Lithuanian researches take partnership in the N-EURO-CAD project, funded by Nordic Genebank for Farm Animals (NGH), were designed to analyse genetic diversity within North European, Baltic and Polish cattle breeds, to estimate relationships and genetic distances between them by using genetic markers (Malevičiūtė, Baltrėnaitė, Miceikiene, 2002). Previous studies of red cell antigens and microsatellites were focused in characterizing the breed and studied relationship between breeds. Therefore, the objective of this study was to use red cell antigen system to assess temporal changes in intrabreed genetic variation of Lithuanian White-Backed cattle under restoration.

Materials and methods. The material consists of data of 66 Lithuanian native cattle born in 1995-2007, tested at the blood typing laboratory of the Institute of Animal Science of Lithuanian Veterinary Academy (IAS of LVA). Lithuanian White-Backed are classified in to five groups. Group 1 composes females collected in the conservation herd from different parts of Lithuania and bred pure with males from group 4 by artificial insemination (AI) (38 animals). In Group 2 included progeny which were born in 2006 and 2007 (23 animals) from females in Group 1. Group 3 – females with unknown pedigree collected from different parts of Lithuania by phenotype (6 animals). Group 4 – males which are from 4 not related genotypes and are used for AI (10 animals) in Lithuania. Group 5 – typical females from another herd which used own bull and no AI (12 animals).

Genetic markers used to characterize the breed were blood groups. Breeds were analyzed by antigenic factors in 2 blood groups system: EAB, EAC. EA refers to Erythrocyte Antigen System. Expected heterozygosities (under Hardy-Weinberg equilibrium) were calculated for each of the groups as $1 - \sum p^2$ averaged over all loci (Nei, 1978). The number of effective alleles (Na) of populations and the genetic similarity (r) between different groups were computed by conventional methods described by Maijala and Lindstrom (1966), Nei (1972). χ^2 - test by Zhivatovski and Machurov (1974) was used for estimating the probability of differences between four groups concerning frequency of alleles.

Results. The data on the blood group frequencies in EAB and EAC systems of different group of LWB cattle are presented in Tables 1 and 2. LWB cattle have an original allelic constitution and differ in common allele frequencies. It was found that within LWB cattle some EAB and EAC alleles are characteristic only in group 4 (bull group) and for some females from different herds of LWB cattle (1, 3, 5). In EAB system no found uniformity of alleles in all four groups was found.

Kuosa et al. (1999) have reported that the genetic analysis of alleles indicated that I_2 allele (EAB system) frequency was dominating (0.1488) whereas in the present investigation it was found just in related Groups 1, 2 and 4. In comparison of EAC alleles frequencies with data of Kuosa et al. (1999) the similar frequencies was X_2 [C_2''] allele in old and present data of all five groups.

Allele C_2C_2'' in EAC system is quite frequent in Group 5 cattle and not found in other groups. The permutation test showed that all groups of LWB cattle were significantly different. The result is important since it shows that cattle from different herds have retained reasonably not high genetic diversity at the biochemical loci.

Average heterozygosities (Table 3) ranged from 0.7819 (EAC genetic system) in Group 3 to 0.923 (EAB) in Group 4 of LWB cattle. High heterozygosities and large number of alleles were observed in all groups. This is consistent with the large number of crossbreed cattle in population of LWB and suggests that the population have been open. The results suggest that there has been very large gene flow among modern-day pedigree breeds of cattle. Higher heterozygosities in Group 3 reflect that most of selected bulls' foundations have gene immigration from commercial breeds. The selected bulls are typical by phenotype but some of them have introgression from 12.5% to 50% of Dutch White-and-Black, or 25-50% Danish White-and-Black or 25% of Holstein cattle breed.

Genetic similarities between different groups were determined using the genetic distance coefficients calculated from the allele frequencies within two blood group genetic systems. The higher genetic similarities (r=0.583) were detected between Groups 1 and 4 and the lowest (r=0.050) between Groups 1 and 5. The influence of selected bulls (Group 4) on progeny from the conservation herd (Group 2) was defined high (r=0.500).

Table 1. **A comparison of Lithuanian White-Backed cattle breeds by prevalent EAB allele frequencies**

Allele	Frequency				
	Group 1	Group 2	Group 3	Group 4	Group 5
B O_2	-	-	-	0.1000	-
$B_2G_2I_2$	-	-	-	-	0.0417
$B_2G_2Y_2G$‘	-	-	-	-	0.0417
$B_2G_2Y_2K$ O‘A_2‘	-	-	-	-	0.0833
B_2O‘	-	-	-	0.0500	-
B_2P‘	-	-	-	-	0.0833
B_2Y_2P‘G‘G‘‘Q‘	0.1053	0.1304	-	0.1000	-
BO_2Y_2D‘	0.0132***	0.0217	-	-	0.2083***
D‘O‘G‘	-	-	-	0.0500	-
G‘‘	-	-	-	0.0500	-
G_2B‘B‘‘T_2	0.0132	0.0217	-	-	-
G_2I_2	-	-	-	-	0.0417
G_2Y_2	-	-	-	-	0.0417
G_2Y_2B‘B‘‘A_2‘T_2	0.0395	0.0217	0.0833	-	-
$G_2Y_2E_2$‘Q‘	0.1842	0.1304	0.2500	0.0500	-
I‘	-	-	-	0.0500	-
I_2	0.0263	0.0435	-	0.0500	-
I_2E_2‘I‘G‘	0.0132	0.0217	-	-	-
I_2E_2‘Q Q‘	0.1447	0.1739	-	0.1000	-
I_2I‘B‘Q‘	-	-	0.0833	-	-
I_2O‘G‘‘	-	-	-	0.0500	-
I_2Q‘	-	-	-	-	0.0250
O_2[A_2‘]	0.0132**	-	0.1667**	-	-
O_2J_2‘K‘O‘	-	-	-	0.0500**	0.0833**
$OI_2Y_2E_2$‘D‘O‘	0.0132	0.0217	-	-	-
P_2	0.1447	0.1087	-	0.1000	-
P_2I‘	0.0263*	0.0217	0.0833*	-	-
Q‘	0.1447	0.1304	-	0.0500	-
Y_2	0.0132	0.0217	-	-	-
Y_2Y‘	-		-	-	0.0833
B-	0.1051	0.1522	0.3335	0.1500	0.0417
Na	8	10	6	5	7
*P<0.05, **P<0.01, ***P<0.001 indicate level of significances, no asterisk indicates no significance.					

Discussion. The observed variations in intrabreed genetic are of interest in demonstrating relatively considerable differences of allele frequencies and heterozygosity in the majority of the groups.

The present material of allele frequencies was based on females and males separately, whereas the old (1999) data of allele frequencies of LWB were obtained by analyzing breeding females. Majala & Lindstrom (1966) have reported that sexual bias in sampling should not hamper the conclusions of the present study.

In 1994, the first decision in setting up conservation schemes for native cattle was to carry forward the existing variability in the breed. Therefore, females from different herds have wide scale variation of alleles. The results of allele frequencies suggest that there has been different gene flow among modern-day pedigree breeds of cattle, and it has resulted in significant genetic differentiation of the groups.

Tapio et al. (2006) studied 35 European cattle populations and found that 19 out of 24 nonsafe breeds added >10% to the diversity of the safe set, whereas the conservation of the Jutland, Estonian Red, Lithuanian Light Grey, Lithuanian White Backed, and Danish Red in Latvia breeds added the least to the genetic diversity. In addition, Tapio et al. (2006) observed that genetically Lithuanian White Backed (n=40) population represents gene pools similar to Black-and-White type cattle breeds. Eastern Finn cattle and Lithuanian White Backed cattle contributed most of the genetic variation (Tapio et al, 2006). In our investigation Lithuanian White Backed cattle have very different phenotype of alleles within breed. Juškienė (2001) studied the pedigree of the LWB cows in 2000-2001, and concluded that there was a certain higher or lower degree of blood infusion from foreign breeds in to LWB cattle.

Lower heterozygosities were observed in Group 2. Differences of heterozygosities between Group 1 and 2 were 0.0065 and 0.0117 between Groups 2 and 4. This is consistent with the small subpopulation size of this group and suggests that the animals are bred pure (there is no migration from other breeds). All cattle (Group 2) were bred pure on the basis of the principles of maintaining

selection. The circular mating scheme by J. Šveistys (1998) was applied for cattle breeding only in the herd of the institute (Group 1 and 2). In this herd the animals are bred with the purpose to maintain various not related genealogical structures of LWB cattle. Besides, cows are inseminated with the selected bull sperm, though there are semen doses collected and stored from 6 white-backed bulls. Therefore, we can make an assumption that the LWB breed could have lost from 0.31 to 0.58% of their heterozygosities over generation and the degree of inbreeding will be higher if new not related to each' other bulls are not found. Kantanen et al. (1999) observed that Nordic breeds could have lost from 1 to 11% of their heterozygosity over a 20-40-year period. Based on this relationship, Gregory et al. (1999) recommended that at least 20 to 25 sires were used per generation. The use of 25 sires per generation would result in a rate of increase in inbreeding of about 0.5% per generation. Šveistys (1985) described that the application of such circular mating schemes with 4 disconnected pedigree animal groups allows to minimize inbreeding. The coefficient of inbreeding (by Wright) amounted to only 6.2% in four generations. On application of similar mating schemes with 8 disconnected pedigree animal groups, the coefficient of inbreeding should amount to only 3.12% in eight generations.

Table 2. **A comparison of Lithuanian White-Backed cattle breeds by prevalent EAC system allele frequencies**

Allele	Frequency				
	Group 1	Group 2	Group 3	Group 4	Group 5
C_2C_2''	-	-	-	-	0.3750
$C_2E\ R_2$	0.0132	0.0217	-	-	-
$C_2E\ X_2$	0.0132	-	-	-	-
$C_2W\ E$	0.0395	0.0217	0.0833		0.0417
$C_2W\ E\ X_2$	0.1447	0.1522		0.0500	0.1250
$C_2W\ R_2$	-	-	-	0.0500	-
$C_2W\ X_2$	0.0921	0.0869	0.1667	0.0500	-
E	0.0132***,**	-	0.0833***	-	0.1250**
$E\ X_2$	0.0132**	0.0217	-	0.1000**	-
$R_2[C_2$'']	0.1842	0.2174	0.0833	0.1000	
R_2L'	-	-	-	-	0.0417
$W\ R_2$	0.1053	0.0435	0.1667	0.1000	-
$W\ X_2$	0.2105	0.2174	-	0.1500	-
$X_2[C_2$'']	0.0395	0.0435	0.1667*	0.1000*	0.0833*
X_2L'	-	-	-	0.0500	0.0417
C-	0.1314	0.1739	0.2500	0.2500	0.1666
Na	7	6	6	7	5
*P<0.05, **P<0.01, ***P<0.001 indicate level of significances, no asterisk indicates no significance					

Table 3. **Average heterozygosities, number of alleles and sample sizes for each group**

Group	No. of animals sampled	Systems	Average heterozygosity	No. of alleles
1	38	EAB	0.8972	213
		EAC	0.8077	166
2	23	EAB	0.9045	128
		EAC	0.7880	96
3	6	EAB	0.8933	26
		EAC	0.7819	17
4	10	EAB	0.9230	46
		EAC	0.7929	32
5	12	EAB	0.8992	71
		EAC	0.8389	42

In our investigation animals from Groups 4 and 5 were collected from different regions of Lithuania. Therefore, heterozygosities in Groups 4 and 5 were higher. Lower similarities among Group 1 and 5 reflected their different geographic origin. Previous studies (Blott, Williams, Haley, 1998) aiming to characterize relationships within the European group of cattle breeds have shown that European cattle breeds represent separate gene pools, and that although there may have been gene flow between breeds it has not been sufficient to prevent the breeds becoming genetically differentiated (Kantanen et al. 2000). In general, the relationships among breeds reflected their geographic origin rather than the agricultural use for which the breeds have been selected (Blott, Williams,

Haley, 1998).

In 2005, the calculated risk status for LWB cattle based upon the effective population size N_e was 15. Thus LWB cattle can be given a risk status category according to the effective population size (Šveistienė et al., 2005). In the nearest future it is necessary to ensure that cattle should be bred by pure breeding following breeding schemes not only in conservation herds but also in private farm herds. With reference to our investigation, it might be expected to get animals with lower introgression of international breeds. It is offspring's of old, typical, with or without pedigree cows inseminated with selected bulls. However, LWB cattle intrabreed genetic variation in the period under restoration will continue to increase, especially if the breed remains not isolated. The animals should have descendent from the parents that are included into the register or herd book of the same breed, but in this case no parentage requirements should be applied to the animals that are used for population restoration in the initial period.

Our investigation reasoning new programme guidelines were worked out for the preservation of the native farm animal genetic resources. The main principles of the new programme: Cattle should have descended from the parents that are included into the register or herd book of the same breed. Exception: no parentage requirements should be applied to the White-Backed cattle that are used for population restoration in the initial period and all purebred bulls or their semen used for breeding should be identified by immunogenetic or DNA methods.

Conclusions

1. Allele frequencies differentiation between groups showed that all groups of LWB cattle were significantly different one from another.

2. Genetic divergence of different groups of the LWB cattle in addition to within population genetic diversity is a result of the combined effects of breeding, geographic origin, the extent of admixture occurring during breed foundation and development.

3. Heterozygosities of LWB cattle were higher and ranged between 0.7929 and 0.923.

4. Decrease of heterozygosities in progeny of conservation herd (Group 2) showed that consequently, to purifying the LWB population the breed could have lost 0.4% of their heterozygosity over few generations.

5. The influence of selected bulls (Group 4) on animals from the conservation herd (Group 1 and 2) was defined sufficiently high (r=0.58 and 0.50).

References

1. Blott S.C., Williams J.L., Haley C.S. Genetic relationships among European cattle breeds. Animal Genetics. 1998. N. 29. P. 273-282.
2. Gregory K.E, Cundiff L.V., & Koch R.M. Composite breeds to use heterosis and breed differences to improve efficiency of beef production. Technical Bulletin. Springfield, Virginia, 1999. N. 1875.
3. Juškienė V. Genetic analysis and milk production of Lithuanian Aboriginal Cattle. Proceedings of the 7th Baltic Animal Breeding conference. Tartu, 2001. P. 46-48.
4. Kantanen J, I Olsaker, L-E Holm et al. Genetic diversity and population structure of 20 North European cattle breeds. The Journal of Heredity. 2000. N. 91 (6), P. 446-457.
5. Kantanen J., Olsaker I., Adalsteinsson S., Sandberg K. Temporal changes in genetic variation of North European cattle breeds. Animal Genetics. 1999. N. 30, P. 16-27
6. Kuosa J. Lithuanian Ash-Grey and White Backed native cattle and their conservation. Conservation of Genetic resources of Indigenous Domestic Animal Breeds. Proceedings of International Conference. Baisogala, 1997. P. 34-35.
7. Kuosa J., Tušas S., Boveinienė B. Immunogenetics characteristics of Lithuanian indigenous cattle (Light-Grey and White-Backed). Animal Husbandry. Scientific Articles. Baisogala, 1999. N. 35. P. 117-123.
8. Lietuvos senųjų vietinių žemės ūkio gyvūnų genetinių išteklių išsaugojimo programa. Vilnius, 1997. 26 p.
9. Litwinczuk Z., Programme of protection of Polish Whitebacks cattle resources. Poland. Lublin. 2002. P. 25.
10. Lush J. L.H. Animal breeding plans. Collegiate Press Ames Iowa. USA, 1945.
11. Maijala, K., Lindstrom G. Frequencies of blood group genes and factors in the Finnish cattle breeds with special regard to breed comparison. Annales Agriculturae Fenniae (Seria Animalia Domestica No. 17) 1966. N. 5. P. 76-93.
12. Malevičiūtė J, Baltrėnaitė L, Miceikienė I. Domestic cattle breed diversity in Lithuania. Veterinarija ir zootechnika. T. 20 (42). 2002. P.87-91.
13. Nei M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics. N. 89, P. 583-90.
14. Nei M. Genetic distance between populations. 1972. Am. Nat. N. 106. P. 283-291.
15. Petraitis J. Vietinių galvijų spalva. Lietuvos TSR Pietryčių rajonų vietinių galvijų biologinės ypatybės, ūkinės savybės ir priemonės jiems pagerinti. Vilnius, 1955. P. 24-25.
16. Sæther N., Vangen O. Motives for utilizing the Black-sided Tronder and Nordland: A native cattle breed in Norway. Animal Genetic Resources Information. 2001. N. 31. P. 15-26.
17. Šveistienė R., V. Jatkauskienė, V. Juškienė. Some aspects of immunogenetics evaluations of progeny of conserved Lithuanian cattle's. Proceeding of the 11th Baltic Animal Breeding and Genetics Conference. Palanga. 2005. P. 75-79.

18. Šveistys J. Lietuvos žemės ūkio gyvūnų genetinių išteklių išsaugijimas. Gyvūnų veislininkystės problemos. Tarptautinės mokslinės gamybinės konferencijos pranešimų medžiaga. Baisogala, 1998. P. 41-43.

19. Šveistys J. 1982. Populiacinio metodo panaudojimas Lietuvos baltųjų kiaulių tipams ir linijoms skurti. LGMTI mokslo darbai. N. 19. P. 46-59.

20. Tapio I., Varv S., Bennewitz J. et al. Prioritization for conservation of Northern European cattle breeds based on analysis of microsatellite data. Conservation Biology 2006. Volume 20, No. 6, P. 1768-1779.

21. Vitkus B. Mūsų raguočių klausimu. Žemės ūkis. Žemės ūkio mokslo žurnalas. Kaunas, 1928; N. 6. P. 401-508

22. Wright. S., 1931; Genetics. Princeton Mass. USA, N. 16. P. 97-159.

23. Мешкаускас Ч.П. 1967. Изучение групп крови у литовских пород скота и их применение в племенной работе. Автореф. канд. дис., Каунас, 20 с.

24. Животовский Л.А., Машуров А.М., 1974. Методические рекомендации по статистическому анализу иммуногенетических данных для использования в селекции животных. Дубровицы, 29 с.

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