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Peculiarities and differences of Žemaitukai and large-type Žemaitukai horse genotypes

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Blood samples from 232 Žemaitukai and 190 large-type Žemaitukai horses were analysed at the Blood Typing Laboratory of the Institute of Animal Science of Lithuanian Veterinary Academy to carry out the blood group and protein polymorphism studies.

Sixteen and 19 alleles in Žemaitukai and large-type Žemaitukai blood samples, respectively, were found in three blood group systems. The corresponding numbers of alleles in three protein polymorphism systems were, respectively, 19 and 20.

In the EAD system, 7 alleles were found for the Žemaitukai breed and 10 for the large-type Žemaitukai breed. Alleles D^{dghm} and D^{ad} are most typical of Žemaitukai and alleles D^{dl}, D^{cgm} and D^{dk} of large-type Žemaitukai. In the EAQ system, the Q^{c} allele typical of both breeds was detected. Alleles D^{cdf}, D^{dfh} and D^{cfgm} were found in the Žemaitukai breed. There were significant differences between the breeds in all (Al, Es, Tf) protein polymorphism systems.

A tendency towards a lower total homozygosity was found in the Žemaitukai breed after comparing the data of 2000 (36.22) and 2008 (33.56). Total homozygosity increased in the large-type Žemaitukai population from 33.41 in 2000 to 35.94 in 2008. Blood group homozygosity in both breeds was lower than that of blood protein.

The study revealed a reduced genetic similarity (r) between the breeds. According to data of 2000 and 2008, the genetic similarity (r) was, respectively, 0.6513 and 0.5785. The genetic similarity between the breeds by the blood group systems was found to be higher (0.8421) and that by the blood protein systems lower (0.6870).

Key words: blood groups, protein polymorphism, genetic similarities

INTRODUCTION

On the basis of historical chronicles, archaeological findings, folklore and the first Lithuanian literature [1] that describes the Žemaitukai, it can be concluded that since the 12th century the breed has been well-known and attributed to Žemaitija (Samogitia). The Institute of the Lithuanian Language and Literature indicates that the term "Žemaitukai" has been widely used in Lithuanian dialects and writings. The literature [2, 3] shows that the Žemaitukai have been known since the 6th–7th centuries. It is considered on the basis of the Old Europe Theory by M. Gimbutienė that the Žemaitukai might be a breed genetically related directly with eastern steppe tarpans which reached the territory of Lithuania together with Indo-Europeans approximately 5 thousand years ago, and that the breed developed together with the Lithuanian nation [4].

For a long time Lithuania had the only horse breed, and that was the Žemaitukai. Even until the middle of the 19th century the Žemaitukai was dominating in the horse breeding of the country. However, the more intensive agricultural development led to the Žemaitukai crossbreeding with different breeds. At the end of the 19th century, lighter and heavier type crossbreds appeared in Lithuania. Target selection, good feeding and housing provided favourable conditions for the development of large-type Žemaitukai and Lithuanian heavy draught horses [5, 6]. Meanwhile, purebred Žemaitukai horses almost disappeared because the breed had been on the verge of extinction even four times.

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From 1959, the old-type Žemaitukai began to be revived by pairing the top heavy-type mares and the Žemaitukai stallion Erelis 3. This stallion was used to consolidate the large-type Žemaitukai and was paired with large-type Žemaitukai mares [6]. The offspring of this combination had distinct traits characteristic of the Žemaitukai. The close family link between of the large-type Žemaitukai and old-type Žemaitukai was shown by the traits characteristic of both types of horses, i. e. endurance, undemanding character, solid build, work efficiency and other characteristics useful in agriculture. During the period 1959–1985, Žemaitukai and large-type Žemaitukai horses were called Žemaitian horses [6].

Large-type Žemaitukai horses are spread in the eastern part of Lithuania, called Aukštaitija. Therefore, there were plans to change the name of this type into the Aukštaičiai breed [7]. However, this plan was not accomplished.

The studies of the phenotypic traits of the Žemaitukai and large-type Žemaitukai [8, 3] allowed to presume these breeds as independent ones. Žemaitukai and large-type Žemaitukai horses are different in their body measurements, indices, growth rate, temperament and working qualities [9]. The average body measurements of the Žemaitukai were: withers height 133.6 cm, chest girth 173.2 cm, body length 142.2 cm, cannon bone girth 17.7 cm. Meanwhile, large-type Žemaitukai measurements were as follows: withers height 151.3 cm, chest girth 189.2 cm, body length 158.4 cm, cannon bone girth 20.7 cm. The differences between the other body measurements were also estimated [9].

At birth, Žemaitukai horses have on the average a 6.2-kg lower birth weight; they are 7 cm lower in height and are born 2 days earlier than large-type Žemaitukai. The horses of both breeds have a compatatively long growing period – 3 to 4 years [9].

The Žemaitukai correspond to the pony horse type by their height and type [2]. However, the wide chest, high indices of extension and massiveness indicate that these horses might develop a high draughting power. Thin, strong legs, the low bony index, round hips, wide trot and good jumping technique, energetic temperament and mobility indicate that Žemaitukai horses are highly suitable for quick trotting and jump riding. Therefore, the Žemaitukai are valued and used as universal horses [9–11].

On the other hand, the body measurements, indices and qualities of the large-type Žemaitukai indicate that these horses are of a harness horse type that can develop high draughting power both at a walking pace and trot. Their temperament is also much quieter than that of the old-type Žemaitukai. The bony index of the large-type Žemaitukai is also higher. These horses are valued as driving horses and used for weighty working and trot [6].

In 1990, both horse breeds were considered as critical ones. Various preservation measures were undertaken and both breeds were included into the FAO lists of protective breeds. The disappearing of the breeds has been stopped, but the breeds are still threatened with extinction (Table 1), especially as far as the narrow genealogical structure of the breeds is concerned.

Žemaitukai and large-type Žemaitukai horses are related geographically and genetically and for a certain period (Soviet times) were considered as two types of one breed. However, studies conducted until now indicate that these horses demonstrate differences in genetics [8, 12–14], historical breed development [6, 15, 16] and phenotypic conformation [11], and that these horses can be considered as belonging to two breeds with a different history, type and purpose.

Genetic studies are very important for disappearing breeds as the results of these studies are used for breeding schemes describing homozygosity in the breed and consequences of blood immigration.

Analysis of genetic markers, such as blood groups and biochemical polymorphisms, allows characterization and evaluation of intra- and interpopulation genetic variability, thus being a useful tool for characterizing and comparing breeds.

The aim of this study was to assess and compare the genetic variability in a sample of Žemaitukai and large-type Žemaitukai breeds by studying three blood groups and three systems of biochemical polymorphism.

MATERIALS AND METHODS

The examination was carried out in the Blood Typing Laboratory of the Institute of Animal Science of the Lithuanian Veterinary Academy. Samples were collected from 232 Žemaitukai and 190 large type Žemaitukai horses. A com-

Table 1. **The status of the Žemaitukai and large-type Žemaitukai breeds**

Breed	Year	Breeding stallions	Breeding mares	Total population	Effective population number Ne	Status
Žemaitukai	1994	4	26	42	13.8	Critical
	2008	40	135	340	123.4	Vulnerable
Large-type Žemaitukai	1994	29	44	164	69.9	Vulnerable
	2008	17	122	260	59.6	Endangered

parison of blood groups and the structure polymorphic systems was carried out. The standard immunological procedure involving hemagglutination and haemolysis [17, 18] was used to detect red cell alloantigens at three blood group loci: EAA, EAD, EAQ [19]. The following alloantigens were available: in EAA – Aa, b, c, d; in AED – Da, b, c, d, l. f. g. h. k. m; in EAQ – Qa, b, c.

The standard methods of PAGE gel electrophoresis [20] were used to identify the following protein loci: transferrin (Tf), albumin (Al), and carboxylesterase (Es).

The frequencies of blood and protein alleles *q, p* and the degree of homozygosity *Ca* were computed by conventional methods as described by Maijala and Lindstrom, Nei, Zhivatovski and Mashurov [22–25]. The frequency of antigenic factors was calculated as follows: $p = n/N$, where p is the frequency of the antigenic factor, n is the number of animals with an investigated antigen, and N is the total number of investigated animals.

The allele frequencies were detected as follows: $q = F/2N$, where q is the frequency of the allele, F is the number of the alleles in the population, and N is the total number of investigated animals.

The homozygosity *Ca* was determined by the following formula:

$$Ca = \sum_{i=1}^{n} q_i^2 ,$$

where n is the number of alleles in the locus, and q_i is the frequency of the alleles.

The genetic similarity between the breeds was determined using the genetic distance coefficients calculated from the allele frequencies within three blood group and three blood protein systems. The genetic similarity (r) between the two horse populations was determined by the following formula:

$$r = \sum_{i=1}^{n} \sqrt{p_i q_i} \quad ,$$

where $p_{i...} p_{n,} q_{i...} q_n$ are gene frequencies in the populations, and r is the genetic similarity coefficient. The maximum genetic similarity equals to 1 and the minimum to 0.

The χ^2 test by Zhivatovski and Machurov (1974) was used for estimating the probability of differences between the two populations concerning the frequency of alleles.

RESULTS AND DISCUSSION

From one breed to another, a total of 35 (Žemaitukai) to 39 alleles (large-type Žemaitukai) were found across the six analysed loci (Table 2). For the whole period considered (2000–2009), changes in allelic frequencies were generally high – higher than 0.04 (large-type Žemaitukai) and 0.06 (Žemaitukai) in the absolute value for most of the alleles.

Homozygosity was generally lower for blood groups than for blood proteins.

Differences between Žemaitukai and large-type Žemaitukai horses were smaller in the protein polymorphism than in the blood group systems.

The allele genetic analysis has indicated that allele A^{ad} is dominant in both breeds ($p < 0.05$), and A^{bc} is most typical of the Žemaitukai (Table 3).

The EAD locus is more informative: 7 alleles were found in Žemaitukai and 10 alleles in large-type Žemaitukai. Alleles D^{dghm}, D^{ad} are dominant in Žemaitukai and alleles D^{dl}, D^{cgm}, D^{dk} in large-type Žemaitukai ($p < 0.05$). In locus EAQ, allele Q^{c} is typical of both breeds. The alleles D^{cdf}, D^{dfk} and D^{cfgm} were not detected in Žemaitukai.

The degree of homozigosity (Fig. 1) ranged from 22.84% (EAD) to 47.09% (EAQ) in Žemaitukai and from 14.35% (EAD) to 46.24% (EAQ) in large-type Žemaitukai horses.

Some differences were observed in the albumin locus (Table 4). Genotype AA is typical of the Žemaitukai and AB of the large-type Žemaitukai ($p < 0.05$). Genotypes FI and II in the esterase locus were dominant in both breeds.

Genotypes SS and RR (Tf) were observed only in Žemaitukai and genotypes FM, FH, MO in the large-type Žemaitukai breed. The genotype FF was dominant in the large-type ($p < 0.05$) and DF in the old-type Žemaitukai ($p < 0.05$) in the transferrin locus. The existing alleles and their frequencies were similar in both breeds.

The degree of homozigosity (Fig. 2.) ranged from 21.63% (Tf) to 53.42% in the Žemaitukai and from 25.35% (Tf) to 60.65% in large-type Žemaitukai. The overall *Ca* was 33.56% for Žemaitukai and 35.93% for large-type Žemaitukai. The breeds are closer in blood groups (0.8241) than in blood proteins (0.6870). The overall genetic similarity *r* is 0.5785 between Žemaitukai and large-type Žemaitukai.

Table 2. **Number of alleles and homozygosity detected at six loci in the horse groups examined**

Horse groups	Blood groups		Proteins		Total	
	No. of alleles	Ca	No. of alleles	Ca	No. of alleles	Ca
Žemaitukai (n = 232)	16	33.12	19	33.99	35	33.56
Žemaitukai (n = 48) [28]	13	31.60	12	42.23	25	36.22
Large-type Žemaitukai (n = 190)	19	30.99	20	40.88	39	35.93
Large-type Žemaitukai (n = 27) [28]	15	31.21	13	35.61	28	33.41

Table 3. **Allele frequencies of blood groups**

Locus	Allele	Allele frequency q			
		Žemaitukai		Large-type Žemaitukai	
		n = 232	n = 48 [28]	n = 190	n = 27 [28]
EAA	ad	0.3772*	0.333	0.4368*	0.4074
	bc	0.1401*	0.1979	0.0526*	0.037
	b	0.082	0.0833	0.1158	0.1111
	c	0.0496	0.0833	0.0579	0.037
	A–	0.3511		0.3369	
EAD	dghm	0.3707*	0.4896	0.1500*	0.2037
	cgm	0.0927*	0.1771	0.2026*	0.0925
	dk	0.0560*	0.0417	0.2026*	0.1296
	dl	0.0366*	0.0104	0.1316*	0.037
	bcm	0.0539	0.0521	0.0658	0.1111
	dkl	0.0108		0.0184	0.074
	ad	0.1509*	0.1042	0.0632*	
	cdf			0.0079	0.0185
	dfk			0.0053	0.037
	cfgm			0.0500	
	D–	0.2284		0.1026	
EAQ	abc	0.0043*	0.0313	0.0263*	0.0925
	bc	0.0388*		0.1053*	
	c	0.3190*	0.3542	0.1947*	0.0185
	b	0.0280	0.0521	0.0184	0.2222
	ac	0.0043		0.0132	
	Q–	0.6056		0.6421	

* $p < 0.05$.

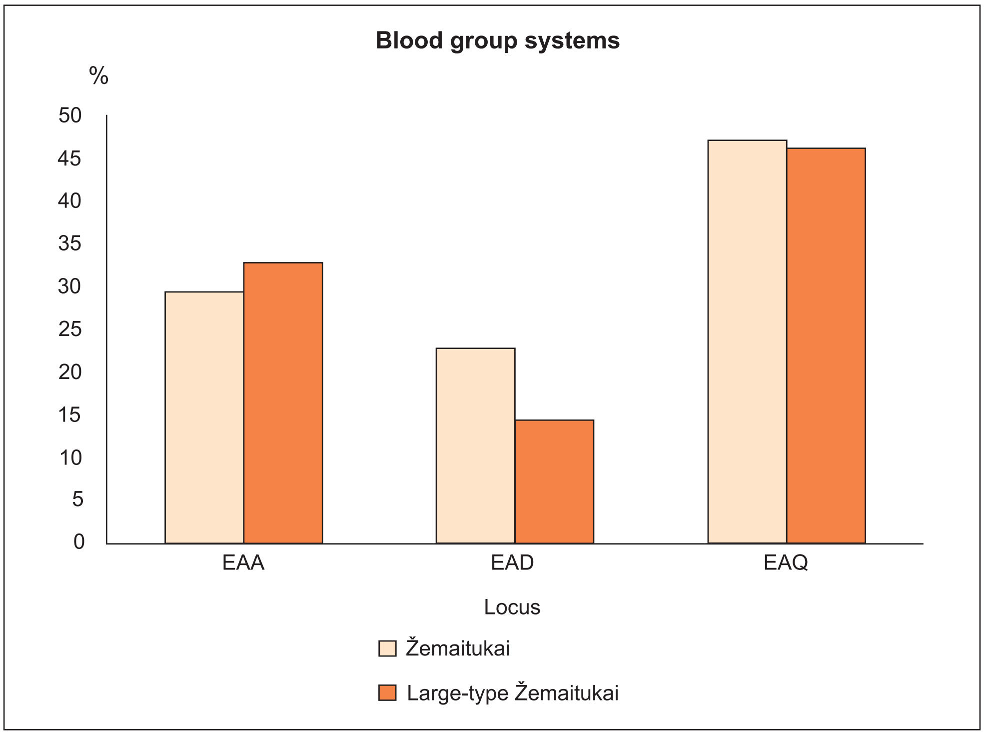

Fig. 1. Degree of homozigosity in blood group systems, %

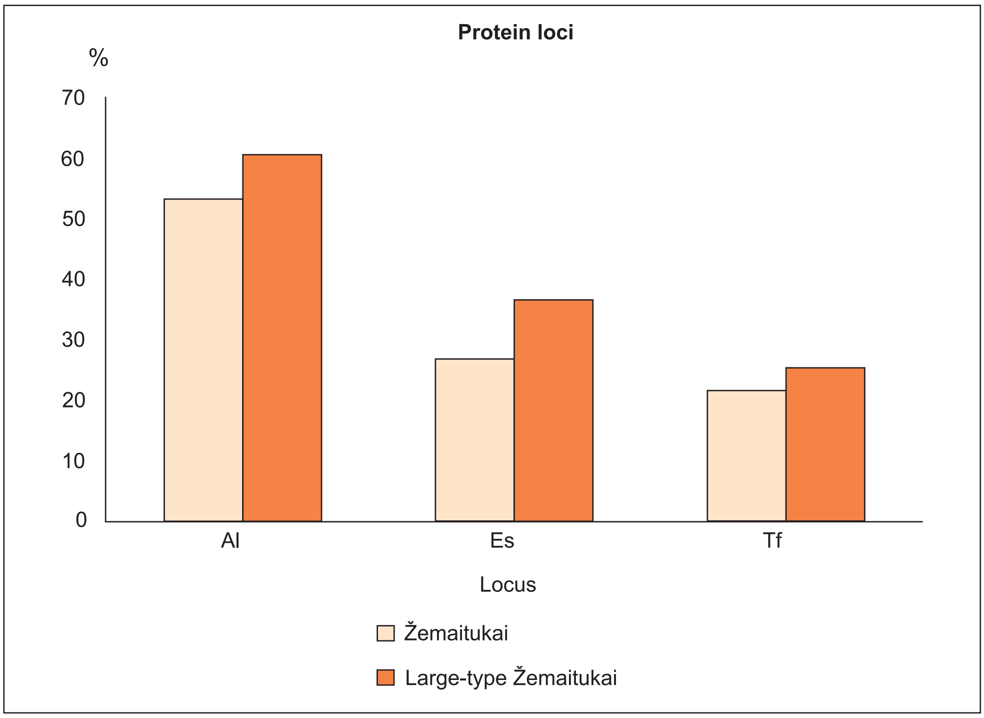

Fig. 2. Degree of homozigosity in protein loci, %

Table 4. **Protein gene frequencies**

Locus	Genotype	Allele frequencies p			
		Žemaitukai		Large-type Žemaitukai	
		n = 232	n = 48 [28]	n = 190	n = 27 [28]
Al	AA	0.6466*	0.6576	0.2632*	0.4444
	AB	0.3405*	0.3243	0.6368*	0.4814
	BB	0.0129*		0.1000*	0.074
Es	FF	0.0474*	0.1621	0,1579*	0.1481
	FI	0.3578	0.2432	0.4158	0.2222
	II	0.3190	0.2116	0.4105	0.5555
	IS	0.1724*	0.1891	0.0053*	0.0741
	FS	0.0862*	0.1891	0.0105*	
	SS	0.0172			
Tf	DD	0.1552	0.1891	0.0053*	
	FF	0.1379*	0.1891	0.4053*	0.6296
	DF	0.3793*	0.4054	0.1684*	0.1481
	DO	0.1336*	0.1621	0.0526*	
	DR	0.0388		0.0105	
	FO	0.0905*	0.0504	0.2211*	0.074
	FR	0.0388		0.0789	
	OO	0.0129		0.0053	
	FM			0.0053	0.037
	FH			0.0210	0.074
	OR	0.0086		0.0210	
	MO			0.0053	
	RR	0.0043			

* $p < 0.05$.

DISCUSSION

The results showed some characteristics shared by the two breeds studied. The genetic disequilibrium observed in the Žemaitukai and large-type Žemaitukai populations could be interpreted as a result of allelic introgression from other breeds when the Žemaitukai population was restored [26, 27].

While comparing Lithuanian breeds with the other breeds, we can find a lot of differences between the breeds. For example, in the Mangalarga Machador and Mangalarga breeds allele D^{dfkl} is specific, but it is not typical of our breeds and in the Žemaitukai population has not been found.

D^{cgm} and D^{dkl} are considered to be specific alleles of the Lithuanian Arabian [28]. The occurrence of these alleles in the animals in this study evidences the influence of this breed on the formation of the Žemaitukai. Jiskrova et al. [29] from the Czech Republic has established that the allele EAD^{dkl} is characteristic of the Czech warm-blooded and Trakehner horse population. The Czech warm-blooded horse, which is very heterogeneous due to the influence of imported breeds, is genetically the least consolidated breed [29].

The lower frequencies of Tf^{FF}, Es^{II}, Al^{AA} and the increasing homozygosity in the protein polymorphism locus in the large-type Žemaitukai and Es^{FF}, Es^{FS} protein polymorphism in the Žemaitukai population support the idea that these breeds can lose some variability in the future.

After World War II, the Žemaitukai breed was restored from the large-type Žemaitukai and one stallion Erelis. Their genetic similarity was high – $r = 0.6513$ [28, 30]. The substructure indicates that the breeds have been uniform genetic entities for many years. There were strict breeding rules, and interbreeding was not prior to herdbook establishment.

The Žemaitukai breed was restored from the sub-lines of Arab, Estonian Native stallions and large-type Žemaitukai during the last ten years [31]. Žemaitukai horses produced by pure breeding account for 85.2% of the population, while 14.8% are representatives of newly developed lines. The development of the new lines of the Žemaitukai horse breed has been carried on since 1994 with Arab stallions and since 1999 with large-type Žemaitukai and native Estonian stallions. Therefore, at present, the genetic similarities ($r = 0.5785$) between Žemaitukai and large-type Žemaitukai are decreasing. Currently, the decreasing relationship suggests an overall differentiation (0.07%) between the breeds. The differentiation of two Lithuanian native breeds could be explained by using different breeding schemes.

Imported horses of Arab and Estonian origin influenced the development of the Žemaitukai breed. However, the allels of blood groups and protein polymorphism suggest a clear differentiation indicating that large-type Žemaitukai have had a little impact on the genetic composition of the Žemaitukai breed.

The results of this study indicate that using the best stallions leads to an increase in selection intensity, but this is not an optimum for small populations of preserved horses. Therefore, the breeders' associations have developed selection programs which safeguard the preservation of the genealogical structure of native populations.

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ŽEMAITUKŲ IR STAMBIŲJŲ ŽEMAITUKŲ ARKLIŲ GENOTIPO YPATUMAI IR SKIRTUMAI

Santrauka

LVA Gyvulininkystės instituto Gyvūnų kilmės patikrinimo laboratorijoje buvo ištirti 232 žemaitukų ir 190 stambiųjų žemaitukų kraujo mėginiai – atlikti kraujo grupių ir baltymų polimorfizmo tyrimai.

Trijose genetinėse kraujo grupių sistemose nustatyta 16 alelių žemaitukų veislės mėginiuose ir 19 alelių stambiųjų žemaitukų mėginiuose. Trijose baltymų polimorfinėse sistemose rastų alelių skaičius atitinkamai – 19 ir 20.

Kraujo grupių EAD sistemoje nustatyti 7 aleliai žemaitukų ir 10 alelių stambiųjų žemaitukų mėginiuose. D^{dghm} ir D^{ad} aleliai būdingiausi žemaitukams, o D^{dl}, D^{cgm} ir D^{dk} aleliai – stambiesiems žemaitukams. EAQ sistemoje nustatytas abiem veislėms būdingas Q^{c} alelis. Žemaitukų veislėje neaptikti D^{cdf}, D^{dfh} bei D^{cfgm} aleliai. Visose baltymų polimorfinėse sistemose (Al, Es, Tf) nustatyti patikimi skirtumai tarp veislių.

Palyginus 2008 m. tyrimų duomenis su 2000 m. skelbtais duomenimis, žemaitukų veislėje pastebimas bendras homozigotiškumo sumažėjimas (36,22 – 2000 m., 33,56 – 2008 m.). Stambiųjų žemaitukų populiacijos bendras homozigotiškumas padidėjo (33,41 – 2000 m., 35,94 – 2008 m.). Abiejų veislių kraujo grupių homozigotiškumas yra mažesnis negu kraujo baltymų.

Tyrimais nustatytas sumažėjęs genetinis veislių panašumas (r). 2000 m. skelbtų tyrimų duomenimis, genetinis panašumas (r) buvo 0,6513, o 2008 m. duomenimis – 0,5785. Didesnis (0,8421) genetinis panašumas nustatytas veislių kraujo grupių sistemose, o kraujo baltymų sistemose – mažesnis (0,6870).

Raktažodžiai: kraujo grupės, baltymų polimorfizmas, genetinis panašumas