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# An attempt at analysing the selected traits of body conformation, growth, performance and genetic structure of Lithuanian native Žemaitukai horse, the breed being preserved from extinction

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Over the last 50 years the traits of valuable indigenous Lithuanian Žemaitukai horse have not been investigated and during the last decade the breed became on the verge of extinction. Recently certain measures were undertaken to preserve the breed, evaluate its present characteristics and compare them with those reported earlier.

Body size and conformation traits of present-day Žemaitukai horse (ŽH) were found corresponding or similar to those of the ancient type, showing that many valuable characteristics of the breed are retained. Mares' milk yield and composition were evaluated, as well as development of foals based on body dimensions.

Genetic variation, genetic structure as well as relationship between the lines and families of  $\hat{Z}H$  were studied using blood typing and electrophoretic analysis of serum proteins. Gene frequencies are presented at six blood group (*A*, *D*, *C*, *Q*, *P*, *K*) and five protein (*Al*, *Es*, *Gc*, *Xk*, *Tf*) *loci*. The genetic diversity within blood groups and serum proteins in  $\hat{Z}H$  kept in a closed population showed that out of eleven genetic systems examined, six were polymorphic. This is especially so for the *A* and *D*, as well as *Es* and *Tf* systems. The distribution of allele frequencies varied between the lines and families.

KEY WORDS: allele / blood groups / breed protection / genetic polymorphism / endangered breed / horse

The Žemaitukai is an ancient indigenous Lithuanian horse breed known since the VI-VII century [Gleß 1989, Aleksa 1951]. The Žemaitukai became especially famous in the XIV century as excellent battle horses during the Lithuanian-Crusader Wars [Petraitis 1948, Buden 1952]. Later, the Žemaitukai developed into draft horse mainly

used in agriculture [Kvašninas-Samarinas and Mockus 1927]. The wars and reforms of the XX century had a devastating effect on the breed. After World War II, efforts were undertaken to restore the breed which in the nineties became on the verge of extinction. In 1994 there existed only 30 purebred Žemaitukai horses [Garbačauskaite 1998].

The Lithuanian Institute of Animal Science (LIAS) has developed the conservation programme for the Žemaitukai breed and took measures to prevent the breed from total extinction. The FAO Mission Conference for Central and East European Countries recognized the Žemaitukai horse as highly valuable and of international interest. The breed was included into the World Watch List for Domestic Animal Diversity [1995].

The Žemaitukai breed should be preserved not only as the national historical and cultural heritage, but also as an invaluable genetic resourse for horse breeding. Žemaitukai horses are the founders of two different horse breeds. In the XVIII century Žemaitukai mares have contributed towards the formation of the Trakehner breed, and in the XX century, the Žemaitukai became the foundation for establishing the Lithuanian Heavy Draught and heavy-type Žemaitukai horse [Garbačauskaite-Macijauskene [2000].

At present the Žemaitukai horse population consists of two stallion lines and five mare families. In 1959 when the Žemaitukai herd was restored at Vilnius Stud, stallion Erelis, the only remaining after war, was mated to it's daughters what led to close or very close inbreeding – 12.4-25% – as estimated according to Wright's formula. In ten years Erelis has sired 45 offspring and at the beginning of 2002, as many as 64 living horses belonged to his line. For over a decade, the Erelis line was the only one sustaining the Žemaitukai breed, composed of closely related individuals. In 1970's to avoid incest, three other stallions were used – Killian 637 and Kipper 638, both of Polish Primitive Horse breed (Konik) and Asturas 634 of Estonian native horse. The results of crossing Žemaitukai with Polish Koniks appeared not inspiring, while from the progeny of Asturas 634 a suitable ancestor of the Žemaitukai breed – Agentas 739 – was selected. The latter has sired a total of 30 offspring and at the beginning of 2002 his line comprised 46 living horses.

Out of five mare families, only the one of Kaštanke 0399 is represented by significant number of individuals. At the beginning of 2002 the family numbered 47 mares and fillies. Within the family the effect of different stallion lines is mostly evident. The progeny of mares with the higher blood share of Asturas 634 has, until now, lighter – yellow, yellow dun or mouse-grey – coat colour at birth than that of Erelis line. Their chest is narrower, legs are longer and head coarser. In turn, the foals with higher blood share of Erelis line are born mostly dark-bay and their general appearance is more elegant.

The family of Mirta is represented by only four mares, but is distinguished by its typical Žemaitukai traits. Valuable stallions – Aruodas 633 and Agentas 795 – have originated from the family. Therefore, the reproduction within the Mirta family is considered important.

The family of Žibute was least numerous, but now it consists of 12 individuals as two mares of the family gave only fillies. The pedigree of the family founder – Žibute – is not exactly known, but the progeny of the family is considered highly typical of

the Žemaitukai breed.

The peculiarity of the family of Arabe 400 is defined by the fact that the features of the Arabian horse used in the past are inherited by these mares in even 4th or 5th generation. The family numbers four mares characterized by the Arabian specific temperament, elegant carriage and dish-nose what is typical only of this family.

The family of Tulpe 411 numbers nine mares and fillies distinguished by coat colour variety, solid constitution and more ordinary head. The mares of the family are least typical of the Žemaitukai breed.

At the beginning of 2002, the Žemaitukai population numbered 110 purebred horses of which 64 belonged to the Erelis and 46 to the Asturas 634 line. Erelis line comprised nine pedigree stallions and 10 non-tested foals, while the line of Asturas 634 comprised five pedigree stallions and 10 foals. The families were represented by 76 mares and fillies of which 47 belonged to the family of Kaštanke 0399, four to Mirta, 12 to Žibute, four to Arabe 400 and nine to Tulpe 411. The genealogical structure outlined did not ensure preserving the breed, and from 1999 the two new stallion lines are being developed in the Žemaitukai pedigree herd belonging to the LIAS.

#### Material and methods

The conservation of the Žemaitukai horse (ŽH) was started in 1994, by establishing the pedigree herd at the LIAS, Baisogala. The horses were bred pure by the method of closed populations in small herds and evaluated for their origin, breed type and body conformation. The following body measurements were taken: height at withers, at back and at croup, oblique body length, chest girth, chest depth, chest width, croup width, length of forelegs, cannon bone girth, head length, forehead width, hoof length and hoof width. Recorded was pregnancy length, growth rate of foals (body dimensions) and milk yield of mares. The growth rate of foals was determined by their measuring at birth and at 1, 2, 3, 6, 9, 12, 18, 24 months of age, and then every six months till the age of four years. The milk production during the first month of lactation was determined as described by Dobrynin [1955] based on the live weight of the foal. Milk yield of mares in five lactation months was estimated based on lactation curves according to Krasnikov [1973]. Milk samples were analysed for dry matter, fat, protein, ash and lactose.

Blood samples were collected from 21 horses from Asturas line, 32 from Erelis line, 30 from Kaštanke family and 6 from Žibute family. Two 10 ml samples were obtained from each horse, one on ACD anti-coagulant and another to a dry tube to be used as a source of red cells and serum, respectively. Standard immunological procedures involving hemagglutination and complement-mediated haemolysis [Stormont and Suzuki 1964] were used to detect red cell alloantigens at six internationally recognized blood group *loci: A, D, C, Q, P* and *K* [Sandberg 1995]. The reagents used to detect the antigenic properties were obtained by alloimmunization. Assignment of alleles was based on reagent reaction patterns and followed the internationally accepted terminology.

Standard methods of horizontal polyacrylamide gel electrophoresis [Juneja et al.

1978] were used to identify inherited variants at the following protein *loci*: albumin (*Al*), esterase (*Es*), vitamin D-binding protein (*Gc*), A1B glycoprotein (*Xk*)) and transferrin (*Tf*). Frequency of antigenic factors, allele frequency, genetic similarity and degree of homozygosity (*Ca*) were computed by conventional methods described by Maijala and Lindström [1966], Rendel [1967], Matousek [1964], Nei [1972], and Zhivatovski and Mashurov [1974].

### **Results and discussion**

According to Kvašninas-Samarinas and Mockus [1927] the body conformation traits of ŽH have been described as early as in 1874 by Tchapsky, in 1899-1902 by Urusov, and in 1912 by Moratchewsky. Later some data were submitted by Kvašninas-Samarinas and Mockus [1927], Žebenka [1934], and Petraitis [1948]. Over the last 50 years, body conformation, biology and working performance of the ŽH horse have not been investigated. The analysis and breed conservation was started in 1994. In spite of the fact that during the last 50 years ŽHs were inbred, they are still considered to have retained most of their valuable traits such as resistance, strong build, agility, adaptability and high fertility.

**Body dimensions** as withdrawn from the earlier publications *vs* those obtained by Garbačauskaite [1997] of present-day ŽHs are given in Table 1. Wither height is 133-138 cm for stallions and 130-136 for mares. The chest girth is 171-187 cm for stallions and 154-187 for mares, and cannon bone girth 18-19 cm for stallions and 16-18 cm for mares. Body weight varies from 360 to 415 kg. Both larger and smaller than optimum stature horses get lower scores at evaluation. It is considered inexpedient to increase the ŽH size, as there also exists the heavy-type ŽH variety, the wither height of which amounts to 145-155 cm. It is concluded that the contemporary ŽH horses resemble their ancestors from the turn of XIX century.

Table 2 presents the body dimensions recorded for ŽH foals, while in Table 3 the respective figures are given as per cent of those recorded in adults aged four years. The foals presented the highest growth rate over first six months of life. Three years old foals reached and even surpassed their parents in height, croup width and length of forelegs but their chest width, chest girth as well as body length were inferior to those of their parents. In relation to the final live body weight the foals grew most intensively during the first month, gaining on average 1.25 kg daily. Horses at the age of 3.0-3.5 years can be used for breeding. In agriculture, however, only the use of fully matured horses, *i.e.* at the age 4-5 years is recommended.

During the first month of lactation the daily **milk yield** of ŽH mares amounted to about 12.7 kg/day leading to a total of about 1770 kg in five lactation months. Mares yielded 30.2 g milk per kg live body weight, what is more than generally reported for light and heavy type horse breeds, but less than in Shetland ponies (Tab. 4). Dry matter content of milk varied from 9.9 to 10.9%, that of fat from 0.48 to 2.0%, of protein from

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Stallions (n = 10)											
Wither height Backheight Rump height Body length Orest depth Orest width Orest girth Cannon bore girth Head length Rochead width Oroup width Rockleg length	899 875 902 668 299 169 781 119 308 146 193 628	99.1 970 1003 79.7 363 209 966 12.7 350 17.1 233 67.2	107.6 104.2 109.0 99.4 43.1 26.2 1139 14.5 42.3 19.2 28.0 70.8	1155 1147 1184 1142 497 293 1327 1327 156 456 209 342 756	1218 1186 1259 1175 524 329 1372 136 476 213 369 772	124.8 121.1 1266 1209 535 343 1412 166 499 21.7 379 793	126.8 124.0 130.8 127.8 58.9 38.9 152.9 152.9 152.9 16.9 52.8 22.2 40.6 79.4	1298 1260 1319 1329 619 419 1630 169 539 225 428 797	131.8 126.9 133.9 62.0 42.9 166.0 17.4 54.9 22.9 44.1 80.0	1338 1280 1365 1400 630 439 1687 178 560 237 449 809	133.1 127.2 134.8 141.3 62.2 45.3 172.2 18.1 56.0 23.7 45.2 80.0
Mares (n= 12)											
Wiher height Backheight Rump height Body length Onest depth Onest girth Carst girth Carst girth Head length Rowhead width Group width Rowleg length	91 5 89 5 92 2 69 6 30 5 17 4 80 5 12 3 33 7 15 0 20 2 64 8	100.1 97.5 101.6 83.2 35.6 21.9 100.0 13.3 37.0 16.8 23.4 68.8	110.4 1066 1126 1016 43.1 27.4 1193 14.7 41.6 18.7 29.0 73.2	1183 114.7 121.2 1130 49.1 330 136.4 15.7 466 20.5 34.8 76.5	123.6 119.5 126.4 120.8 52.4 33.9 140.8 15.9 48.7 21.0 37.2 78.2	1255 1216 1286 1242 546 348 1468 162 159 214 388 796	130 2 1260 1339 1327 396 1390 172 532 223 419 814	1323 1278 1353 1352 598 405 1626 173 537 227 448 816	132.8 128.1 135.9 137.8 60.0 42.0 165.3 15.7 54.6 23.0 45.1 81.6	134.1 1292 1378 1404 619 43.1 1728 176 549 234 455 816	133 <i>1</i> 128 <i>8</i> 136 <i>8</i> 142 <i>4</i> 63 2 46 2 179 <i>7</i> 17 <i>8</i> 55 <i>6</i> 23 <i>8</i> 45 2 81 0

Table 2. Body dimensions (cm.) of Žemaitukai foals

\*Parents of foals considered.

1.9 to 3.0%, of lactose from 6.6 to 8.4, and of ash from 0.27 to 0.49% (means were 10.2, 1.3, 2.3, 7.2 and 0.38%, respectively), figures not included in Tables.

**Coat colours** were found characteristic of the ŽHs. Currently 43% of the horses are bay, 21% black, 19.1% chestnut, 9.6% dark-bay, 4.6% mouse-grey, 3.8% yellow dun. Large white marks and piebald coat colours are not characteristic of ŽHs.

ŽHs are noted for their harmonious body proportions, excellent conformation, smooth and elegant carriage, strong trot, energetic yet compliant disposition. This is an all-purpose breed suitable for work on small farms, driving and riding. The horses show excellent endurance in long distance races. Due to small size and easily manageable character, ŽHs are very suitable for children's sports and tourism. In the future the

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Stallions (n = 10)											
Wither height Backheight Rump height Body lergth Orest depth Orest width Orest girth Carnonbone girth Head length Rochead width Group width Rocheg length	67.6 68.8 66.9 47.3 48.2 37.5 45.4 66.2 55.0 61.6 42.7 78.6	74.5 763 74.4 56.4 463 56.1 702 626 72.1 51.5 84.0	809 819 809 704 693 580 662 801 757 810 620 885	869 902 879 808 800 646 77.1 867 816 886 758 946	916 933 934 832 943 728 797 900 850 898 818 966	939 952 939 856 861 757 820 922 892 919 840 991	95.4 97.5 97.1 90.5 94.7 86.0 88.8 93.9 94.4 93.6 90.9 90.9 90.9	97.6 99.1 97.9 94.1 99.6 92.7 93.9 94.7 93.9 94.7 94.7 90.7	99.1 99.8 99.4 96.2 99.8 94.9 96.4 96.6 98.2 97.0 97.5 100	100.6 100.7 101.3 99.1 101.3 97.1 98.0 98.3 100.0 100.0 99.5 101.2	100 100 100 100 100 100 100 100 100 100
Mares (n= 12)											
Wiher height Backheight Rump height Body length Chest depth Chest width Chest girth Canan bone girth Head length Rochead width Croup width Rocheg length	68 5 69 5 67 4 48 9 48 2 37 7 44 8 69 1 60 6 63 2 44 7 80 1	749 757 743 584 564 475 556 747 667 708 519 850	82.6 82.8 82.3 71.4 68.2 99.4 66.4 83.1 74.8 78.7 64.2 90.4	885 891 886 794 777 715 759 882 834 863 770 944	925 928 924 849 830 735 784 893 877 883 824 966	939 944 940 872 864 754 817 915 917 900 860 983	97,4 97,9 93,2 90,6 85,9 88,5 96,6 95,8 93,8 93,8 92,8 100,6	99.0 99.3 98.9 95.0 94.7 97.1 96.7 95.5 99.3 100.8	99.4 99.5 99.4 96.8 95.0 91.1 92.0 98.3 98.2 96.7 99.7 100.8	1003 1003 986 981 962 962 988 989 984 1007 1008	100 100 100 100 100 100 100 100 100 100

Table 3. Body dimensions (%) of Žen aibibai foals as related to those of adults

Selected traits of Lithuanian Žemaitukai horse

\*Parents of foals considered.

Table 4. There htimship between milk yield and body weight of mares

Bræd	Mean) infi	hilymik yield remarihs af hatation	Men	i body weight	Milk yield (glegbody
	kg	% of Shetland	kg	% of Shetland	weight)
		DERV		DEW	
Ženaitukai	11.8	104	390	177	30.2
Heavy-type Žemainikai	14.7	129	520	236	28.2
Heavy-type horse <sup>1</sup>	17.1	150	710	323	24.1
Light type harse <sup>1</sup>	14.3	125	590	263	24.2
Shetlandpary <sup>1</sup>	11.4	100	220	100	52.0

Šveistiene [2000]. <sup>1</sup>Shavatk [1978].

#### ŽH should become a Lithuanian family horse.

The conservation of the breed was started in 1994 by forming the pedigree herd at the LIAS, Baisogala. The genealogical structure of of the ŽH breed is being formed by pure breeding according to circular mating scheme in small herds. In order to avoid the total loss of the breed, the breeding nucleus is currently concentrated in two herds: one belonging to LIAS, Baisogala, and the other to Vilnius Stud. Several smaller herds are being formed by country life museums, agricultural schools and individual holders. In 1997 Žemaitukai Horse Breeder's Association was established.

Repors on gene frequencies within blood group and serum protein systems in ŽH are very few [Boveiniene and Jatkauskiene 1998, Boveiniene et. al. 2000a, 2000b, Hamanová et al. 2001]. The characteristic blood group alleles, serum protein alleles and the degrees of homozygosity obtained in this study are shown in Table 5. The most effective *loci* were those having five or more alleles with appreciable frequencies, namely D and Tf. The genetic analysis of alleles indicated that  $D^{dghm}$  allele was typical of ŽH, alleles  $D^{adl}$ ,  $D^{cgm}$  and genotype  $Tf^{DF}$  were also frequent. The degree of homozygosity ranged from 19.1% (*Tf locus*) to 100% (*Xk locus*), with mean value of 49.2%. Tables 6 and 7 show the frequencies of alleles within blood group and serum protein systems occurring in the horses of Asturas and Erelis lines and Kaštanke and Žibute families. A<sup>cd</sup> allele in EAA system was detected only in Asturas line. Analysis of allele and genotype frequencies on which the estimation of genetic similarity between the lines and families should be based showed that the frequencies of alleles were distributed from 0.0156 to 1. The most frequent allele at D system was  $D^{dghm}$  (frequency 0.42-0.50). Within the Tf system four alleles -D, F, O, R – have been found. Allele R appeared only in Erelis line and Kaštanke family, while D showed higher frequency (0.56) in Erelis line and the lowest in Zibute family (0.16). A comparison of allele frequency of esterase *locus* showed that allele F was more frequent in Asturas line (0.55) and Žibute family (0.58), and allele I more frequent in Erelis line (0.59). Considering the whole  $\dot{Z}H$  population analysed, the highest frequencies were found for  $Al^A$ ,  $Gc^F$ ,  $Xk^K$  and  $D^{dghm}$  alleles.

**Genetic similarities** between Asturas and Erelis lines, or Kaštanke and Žibute families were determined using the genetic distance coefficients calculated from the allele frequencies within two blood group and five serum protein systems. The genetic similarity between Asturas and Erelis lines was found r = 0.707 while between Kaštanke and Žibute families r = 0.442 (figures are not included in Tables).

The data presented here may be summarized as follows. The former genealogical structure of the Žemaitukai breed (two male lines and five female families) could not ensure the survival of the breed. Blood group and serum protein systems in Žemaitukai horses are polymorphic. This is especially so for the A and D blood group, and Es and Tf protein systems. Allele frequencies vary with the lines and families. The  $A^{cd}$  allele was observed only in Asturas line, while allele  $Tf^{R}$  only in Erelis line and Kaštanke

Lonis	Allele	Allah ingunay (n=3)	Locus	Genotype	Allala fing wanzy (h=73)
Bhod z	n ma mada se		Pro tein :	marlege	
	ф	0.3343		ЬЬ	0.767
	be -	0 199	42	AB .	0.233
Ы	e -	0.082		<u>FF</u>	0.000
	6	0.042	<u>Ca</u> *	64.3%	
	<u> </u>	0.294		FF	0.094
<u>Ca*</u>	26.9%			FL	0.356
	dghm	0.418		11	0192
	dgm	0151	B	FS	0 1 37
	dk	0.027		JS	0 205
	dad'l	0 103		<u>.55</u>	0.014
<i>D</i>	d1	0.021	<u>Ca</u> *	23.5%	
	ðem -	0.055		FF	0.630
	d12	0.014	E2	FS	0370
	ø	0 <u>711</u>		<u>.55</u>	0.000
<u>Ca*</u>	25.8%		<u>Ca</u> *	53.4%	
-	¢	0.473		FF	0.000
L	<u> </u>	0_327	Xk	KK -	1.000
<u>Ca*</u>	50.2%			<u>_KS</u>	0.000
	6	0.048	<u>Ca</u> *	100	
0	e -	0.267		DD	0 2 <b>71</b>
×	be -	0.068		FF	0104
	<u> </u>	0.07		DF	0 <i>19</i> 9
<u>Ca*</u>	45.9%			DO	0109
0	6			DR	0.026
· ·	<u>P</u>		π	FH	0.000
<u>Ca*</u>	91.2%			FW	0.000
ĸ	¢.	0.048		FO	0104
21	K	0.992		FR	0.013
<u>Ca*</u>	90.8%			DH	.013
				00	0.038
				OR	0.013
			代表書	101%	

Teb le5. Game frequencies of blood group and servine protein markers in Zemairul ai horse

Selected traits of Lithuanian Žemaitukai horse

## \*Homosygosity.

family. The genetic similarity between Asturas and Erelis line (0.707) was found higher than between Kaštanke and Žibute family (0.442).

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System	alallA	Asturas line da = 21)	Enclis line (m= 32)	Kastarika <u>family/n=30)</u>	2 into family dn = 6)
A	A <sup>rd</sup>	03333	03125	0.2667	0.5000
	A	0.2837	0.1875	0.2167	0,000
	a <sup>c</sup>	0.0714	0.0312	0.1166	02300
	هم	0.0476	0.0625	0.0660	0,000
	A.	0.0258	0.0312	0.0333	0,000
	A	0.0258	0,000	0,000	0,000
	<u></u>	02143	0.3750	0.3000	02500
Ca*		24.7%	27.9%	22.7%	37_5%
٥	D <sup>rama</sup>	0,000	0,000	0,000	0.0833
	<sup>4254</sup>	0.4762	0.4844	0.4166	0.5000
	off .	0.0714	0.1.562	0.1166	0.0833
		0.0932	0.1250	0.1166	0.0833
		0.1428	0.0781	0.1666	0,000
	ofii	0.0476	0.0156	0.0333	0.1667
	o <sup>e</sup>	0.0258	0.0625	0,000	0,000
	<u>ď</u>	0.1428	0.0781	0.1500	0.0833
Ca*		28.5%	29.1%	25.2%	30.6%

Table 6. Gave frequencies inblood group systems in Žemainikaihorse

\*Homosygosity.

Table 7. Allele frequencies in five serun protein systems in Ženaitskaihorse

Loaus	Allele	Asturas line (n = 21)	Erelis line in = 32)	Kašarke <u>fanilvín=301</u>	Žibute family (n=6)
AL	A	0.8571	0.8.594	0.7667	0.8333
Ca*		755%	758%	64.2%	72.2%
G	FS	0 <i>97</i> 37 0.0 <b>2</b> 63	0.6897 0.3.103	0.7885 0.2115	0.8333 0.1667
Ca*		94.9%	57.2%	66.6%	72.2%
B	F I S	0.5476 0.1905 0.2619	0.1563 0.5937 0.2 <i>5</i> 00	0 2333 0 3333 0 4333	0 <i>_5</i> 833 0.4167 0,000
Ca*		40.5%	439%	35.3%	51.4%
X.	X S	1.000	1.000	1.000 0.000	1.000 0.000
Ca*					
IJ	D F O R	03810 0.4762 0.1428 0.000	0.5625 0.2187 0.1563 0.0625	0.4833 0.2666 0.1833 0.0667	0.1667 0.6666 0.1667 0.000
Ca*		39.2%	393%	34.3%	50.0%

\*Han ozygosity.

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# Próba analizy wybranych cech współczesnych koni litewskich rasy Žemaitukai

#### Streszczenie

Zestawiono dostępne dane o liniach i rodzinach koni żmudzkiej rasy Žemaitukai (mierzyny), ich pokroju, mleczności i składzie mleka klaczy oraz o strukturze genetycznej. Polimorfizm genetyczny scharakteryzowano na podstawie sześciu układów grupowych krwi i pięciu białek surowicy krwi. Największy polimorfizm stwierdzono w obrębie układów A i D grup krwi oraz Es i Tf surowicy krwi.