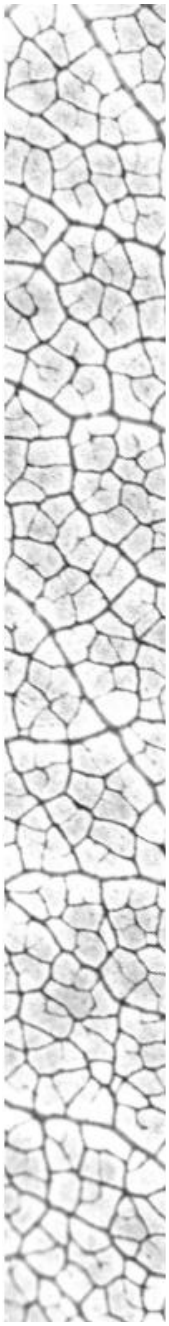

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The distribution of lethal Holstein haplotypes affecting female fertility among the Russian Black-and-White cattle

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Abstract

Background: Many genetic defects have been discovered and proved to be associated with the decrease of female fertility of dairy cattle. These defects are now selected against in many countries. The occurrence of these defects among the Russian dairy cattle has never been extensively screened before. In this study, we will investigate the occurrence of 10 lethal recessive Holstein haplotypes HH0 (BY), HH1, HH2, HH3, HH4, HH5, HCD, HHC (CVM), HHB (BLAD) and HHD (DUMPS) among the Russian Black-White cattle, and we will determine the carrier frequency and the frequency of the mutant alleles for these haplotypes.

Materials and Methods: 1991 animals (1500 bulls and 491 cows) of the Russian Black-and-White cattle were included in the study. Two methods were used for identification of carriers of mutations. The first one is genotyping the animals using the bovine DNA chips that contain the mutations and the second one is the polymerase Chain Reaction (PCR).

Results: The results clearly showed the presence of these defects among the Russian Black-and-White animals. The occurrence was relatively high for some defects such as BY, HCD, HH1 and HH3 with a carrier frequency of 4.11, 5.66, 2.96 and 2.88%, respectively. No carriers were detected for DUMPS.

Conclusions: This work represented an important attempt for extensively screening the occurrence of various genetic defects among the Russian dairy cattle. This has a significant importance in breeding programs. It helps breeders to make an appropriate mating decision by mating only carriers to non-carriers. Such management leads to avoid the economic losses and to decrease the frequency of the mutant alleles in the cattle population.

Keywords: cattle, fertility, genetic defects, Holstein haplotypes

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INTRODUCTION

In dairy cattle, female fertility has been rapidly declining since around 1980 (Barbat et al. 2010). This decrease is a major concern in modern dairy cattle industry, causing a major economic loss for breeders (Fouz et al. 2011, Khatib et al. 2009). Genetic studies have made a great progress towards understanding the genetic factors contributing to decreasing in fertility (Gajbhiye et al. 2018). These studies have resulted in identification of several recessive disorders associated with the decrease in fertility (Sonstegard et al. 2013). Brachyspina syndrome (BY) is one of the genetic defects that reduces the fertility in Holstein cattle. It causes embryonic death, stillbirth and other deformities. It is characterized by a severely reduction of the birth weight of calves despite a normal or a slightly prolonged length of the gestation period, shortening of the cervical and thoracic parts of the spine, long and thin limbs and malformation of the testicles, kidneys, and heart (OMIA 000151-9913). Complex vertebral malformation (CVM) is a hereditary lethal disease in Holstein cattle that results in aborted fetuses and in prematurely born, stillborn, and neonatal calves (Duncan Jr et al. 2001). 77% of CVM affected embryos were aborted prior to gestation day 260 (Berglund, Persson, & Stalhammar, 2004). Bovine leukocyte adhesion deficiency (BLAD) characterized by recurrent bacterial infections, delayed wound healing and stunted growth. Cattle affected with BLAD die at an early age due to the infectious complications (NAGAHATA, 2004). Deficiency of uridine monophosphate synthase (DUMPS) causes the

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Table 1. Description of lethal haplotypes including in this study and the availability of causal mutation tests

Haplotype	Chromosome	Gene	Map location (bp)	Type of mutation	Type of test
HH0	21	FANCI	21,184,869 – 21,188,198	3.3 Kb deletion	causal mutation
HH1	5	APAF1	63,150,400	C>T	causal mutation
HH2	1	–	94,860,836 – 96553,339	-	haplotype
HH3	8	SMC2	95,410,507	T>C	causal mutation
HH4	1	GART	1,277,227	A>C	causal mutation
HH5	9	TFB1M	92,350,052 – 93,910,957	138 kbp deletion	causal mutation
HCD	11	APOB	77,953,380 – 78,040,118	1.3kb insertion	causal mutation
HHC	3	SLC35A3	43,412,427	G>T	causal mutation
HHD	1	UMPS		C >T	causal mutation
HHB	1	ITGB2	145,119,004	A>G	causal mutation

embryonic and fetal mortality in cattle (Ghanem et al. 2006). It leads to loss of homozygous affected fetuses at around the day 40 of pregnancy (Schwenger et al. 1993).

However, many of other genetic defects leading to reducing fertility in dairy cattle were identified after the year 2011. VanRaden et al. (2011) suggested that lethal haplotypes can be discovered by examining haplotypes that had a high population frequency but were never homozygous in live animals. Using this method has resulted in identification of five lethal haplotypes HH1, HH2, HH3, HH4 and HH5 in the Holstein cattle. Further studies have resulted in identification of the causative mutations for HH1, HH3, HH4 and HH5, while the causative mutation for HH2 is still unknown (Cole et al. 2016). Kipp et al. (2015) discovered another Holstein haplotype associated with cholesterol deficiency (HCD). Affected calves showed chronic diarrhea and insufficient development and they died within the first months of life despite of symptomatic treatment. However, many other lethal haplotypes, have been uncovered in Holstein cattle, but the molecular genetic bases for several of them are still unknown (Schütz et al. 2016).

The main dairy cattle breed in Russia is the Russian Black-and-White, which constitutes about 65% of the total Russian dairy cattle population. This breed was created as a result of a cross-breeding between the Russian local dairy breeds and the Dutch Holstein-Friesian. Until now, the occurrence of genetic defects among the animals of this breed has never been extensively screened before. It is expected for these defects to exist in the Russian dairy cattle since the semen, embryos and live animals are still imported to Russia from different countries in order to improve the productivity of the local animals. In this study, we will investigate the occurrence of 10 lethal recessive Holstein haplotypes HH0 (BY), HH1, HH2, HH3, HH4, HH5, HCD, HHC (CVM), HHB (BLAD) and HHD (DUMPS) among the Russian Black-White animals, and we will determine the carrier frequency and the frequency of the mutant alleles for these haplotypes.

MATERIALS AND METHODS

A total of 1991 animals (1500 bulls and 491 cows) from different parts of the Russian Federation were included in the study. The animals were tested for ten lethal recessive haplotypes HH0 (BY), HH1, HH2, HH3, HH4, HH5, HCD, HHC (CVM), HHB (BLAD) and HHD (DUMPS). Information about these haplotypes is provided in **Table 1**.

Two methods were used to identify the carriers of mutations. The first one is genotyping animals using bovine DNA biochips that contain the mutations, and the second one is the polymerase chain reaction (PCR). Based on the availability of genotyping data, animals could be divided into two groups; animals with genotyping data, and animals with no genotyping data. Each of the animals with a genotyping data was examined for all of the 10 mutations of the considered haplotypes, while not each of the animals with no genotyping data was examined for all of the 10 mutations, but at least for one of them.

The biological materials – blood, semen, hair or skin were collected from the animals. Genomic DNA was extracted using QIAamp® DNA Mini Kit (QIAGEN) following the manufacturer protocol. Animals in the first group were genotyped using two different Bovine biochips: Illumina BovineSNP50 v2 and Weatherbys Scientific Bovine VersaSNP 50K.

Illumina BovineSNP50 v2 does not detect the mutations, but it allows detecting of the corresponding haplotypes. All the animals that have been genotyped using this chip, and have been detected to be carriers of a certain haplotype, were examined for the presence of the casual mutation of this haplotype using the PCR method to confirm the carrier status. Animals that were not carriers of a haplotype were assumed to be not carriers of the corresponding casual mutation. The Weatherbys Scientific Bovine VersaSNP 50K includes the mutations for HH1, HH3, HH4, BLAD and DUMPS, but it does not include the mutations for BY, HH5, HCD and CVM, so the mutations of BY, HH5, HCD and CVM were traced using the PCR method for all of the animals that were genotyped using this chip.

The causal mutation for HH2 is still unknown, so recessive test for this genetic disorder was carried out for all the genotyped animals based on the carrier status of the haplotype and not for the mutation. The PCR method was also used to trace the mutations for all the animals in the second group that do not have a genotyping data. **Table 2** illustrates the number of genotyped and non-genotyped animals included in the

Table 2. The number of animals tested for the casual mutations for each of the 10 studied lethal haplotypes based on the method used to detect the mutation

	Type of chip	Total	BY	HH1	HH2*	HH3	HH4	HH5	HCD	CVM	BLAD	DUMPS
Genotyped animals	Illumina BovineSNP50 v2	397	397	397	397	397	397	397	397	397	397	397
	Weatherbys Scientific Bovine VersaSNP 50K	818	818	818	818	818	818	818	818	818	818	818
Non genotyped animals	-	776	683	306	-	314	274	218	446	482	483	402
Total	-	1991	1898	1521	1215	1529	1489	1433	1661	1697	1698	1617
Males		1500	1411	1114	879	1123	1105	1027	1201	1220	1221	1183
Females		491	487	407	336	406	384	406	460	477	477	434

Table 3. Primer sequences, restriction enzymes and size of produced fragments

Haplotype	Primer sequences	Restriction enzymes	Size of fragments (bp)	
			Carrier	non-carrier
BY	AcrossDEL_UP1: GCTCAAGTAGTTAGTTGCTCCACTG	–	409	–
	AcrossDEL_DN1: ATAAATAAATAAAGCAGGATGCTGAAA			
	WithinDEL_UP1: TCACAAAAGGGTAGGAGACTACCTG	–	–	537
	WithinDEL_DN1: GCTTATTGTTTACCCTTGACAGTGG			
HH1	HH1 F: TATAGACTGTGAGAATTTCCAGG	BstMW1	139+17	127+12+17
	HH1 R: TTATCGACCTCCTGCTTGACCTGC			
HH2*	–	–	–	–
HH3	F: AGATTTGCTTCAGCGCTTTGGCAAAAAGTGGTAAAAT	TaqI	151+59+38	151+97
	R: TCTGAGGCTCTTTTTGGTTCTTACCTGAGAATGTGTC			
HH4	HH4_GART_F : GAAGGTGTCCTCTATGCTGG	True9 I	154+117+56	154+59+58+56
	HH4_GART_R: AAGTGCAGAGCAAGCCATCT			
HH5	HH5-TFB1M.F: AGATATGCTAAAGTTTACCTAGAAGAA	–	–	442
	HH5-TFB1M.WT.R: CTGAAGCTCCATTCTGAGTCAT			
	HH5-TFB1M.F: AGATATGCTAAAGTTTACCTAGAAGAA		256	–
	HH5-TFB1M.Del.R: TGCTCTATGAATTTTGTGAATGGT			
HCD	HCD WF: GGTACACATCCTCTCTCTGC	–	–	249
	HCD WR: AGTGGAACCCAGCTCCATTA			
	HCD MF: CACCTTCCGCTATTCGAGAG		436	–
	HCD WR: AGTGGAACCCAGCTCCATTA			
CVM	CVM F: CACAATTTGTAGGTCTCATGGCA	Zsp2 I	287+268+19	287
	CVM R: CGATGAAAAAGGAACCAAAAGGG			
DUMPS	DUMPS F: GCAAATGGCTGAAGAACATTCTG	AvaI	89+53+36+19	53+36+19
	DUMPS R: GCTTCTAACTGAACTCCTCGAGT			
BLAD	BLAD F: GAATAGGCATCCTGCATCATATCCACCA	TaqI	354+198+156	198+156
	BLAD R: CTTGGGGTTTCAGGGGAAGATGGAGTAG			

study, and the number of tested animals for each haplotype.

PCR was performed in a 10 µl final volume, containing 1 µl of DNA template, 0.4 µl each of forward and reverse primers, 2 µl of a ready PCR-mix (Dialat 5X MasCFETaqMIX -2025) and 6.2 µl of H2O. All the reactions were conducted in the T100 ™ Thermal Cycler (BioRad) under the following conditions: 3 minutes at 94°C, followed by 35 cycles each consisting of 30 s at 94°C, 30 s at 58°C and 30 s at 72°C, ending with 5 minutes at 72°C. For HH4 another PCR conditions were used: 3 minutes at 95°C, 35 cycles at 95°C for 30 s, 64 °C for 30 s, 72 °C for 30 s and a final step of 5 min at 72 °C. DNA of known animal carriers of mutations - except for the DUMPS - was used as a control to verify all steps of PCR process.

The method described by Charlier et al. (2012) was used to identify the carrier status of mutation for BY using two pairs of primers. The first pair amplifies the mutant allele (409bp), while the second pair amplifies the wiled type allele 537bp. Three primers were used for screening HCD and HH5 according to the methods described by Menzi et al. (2016) and Schütz et al. (2016), respectively.

Table 4. Total animals, total carriers and proportion of carriers of genetic defects among the Russian Black-and-White cattle

Total animals	Total carriers	Carriers for one monogenetic defect	Carriers for two monogenetic defects	Proportion of carriers %
1991	355	306	49	17.8

The PCR products of BY, HH5 and HCD were analyzed by electrophoresis on a 4% agarose gels stained with a 0.001% ethidium bromide solution. PCR products for HH1, HH3, CVM, BLAD and DUMPS were firstly enzymatically digested, and then the digested products were visualized on 4% agarose gels. The DNA Ladder M-50 (DIALAT Ltd.,cat. no. MWM-50RL) was used to determine the size of fragments. All of the used restriction enzymes, primers and the size of fragments for carriers and non-carriers animals are shown in **Table 3**.

RESULTS

355 animals of overall 1991 animals included in the study have been detected to be carriers of genetic defects. 306 of them were carriers for one defect, while 49 were carriers for two. The proportion of carriers in the

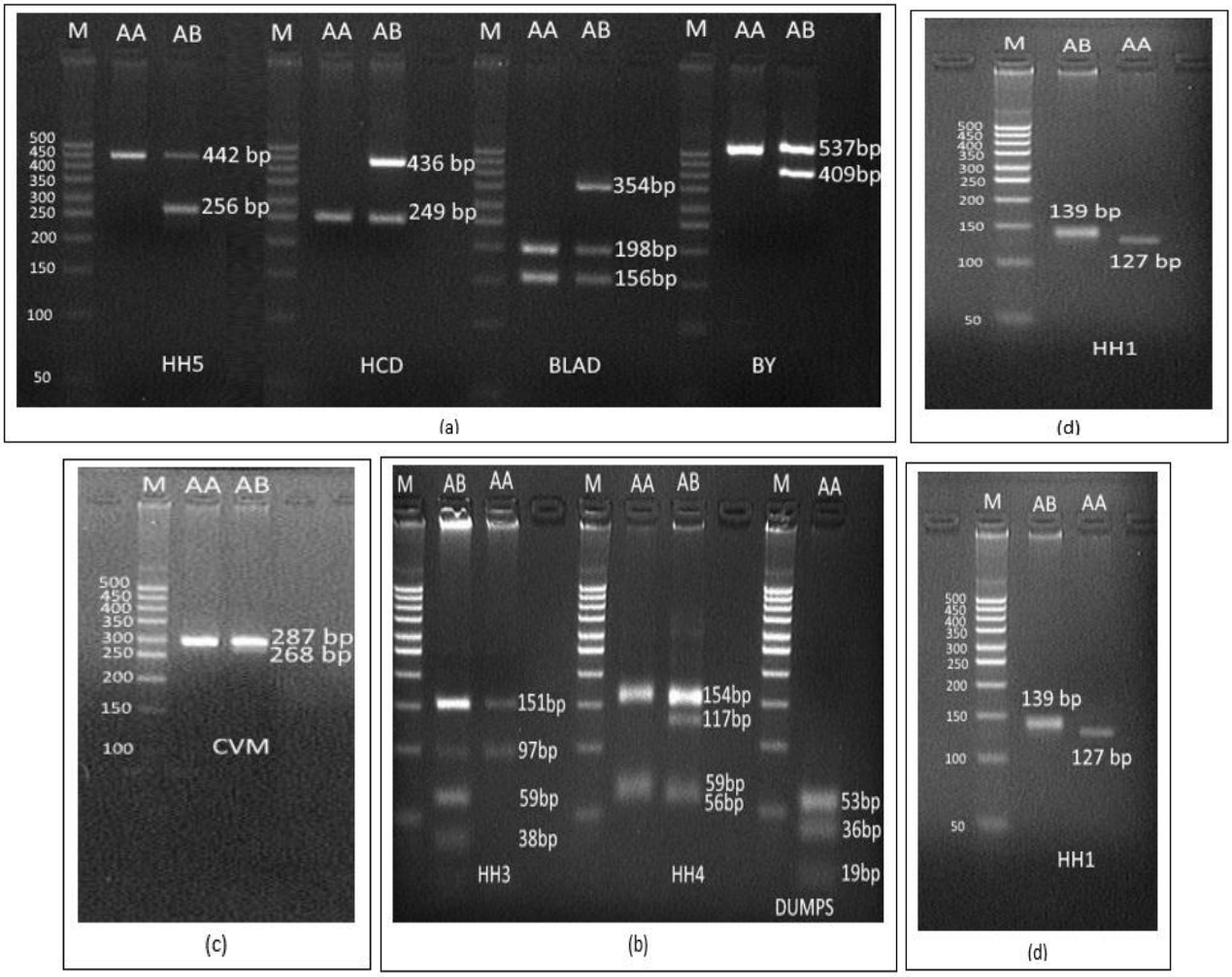

Fig. 1. (a), (b), (c) and (d) show the agarose gel electrophoresis of the PCR products for BY, HH5 and HCD, and of the PCR products after the enzymatic digestion for HH1, HH3, CVM, BLAD and DUMPS. No carriers were detected for DUMPS

population was 17.8% (**Table 4**). The agarose gel electrophoresis of the PCR products for BY, HH5 and HCD, and of the PCR products after the enzymatic digestion for HH1, HH3, CVM, BLAD and DUMPS is illustrated in the **Figs. 1a**-**1d**.

The total number of tested animals for BY, HH1, HH2, HH3, HH4, HH5, HCD, CVM, BLAD and DUMPS was 1898, 1521, 1215, 1529, 1489, 1433, 1661, 1697, 1698 and 1617, respectively. The analysis have resulted in identification of 78, 45, 12, 44, 17, 32, 94, 17 and 16 carriers for BY, HH1, HH2, HH3, HH4, HH5, HCD, CVM and BLAD, respectively. No carriers were detected for DUMPS. That means that the proportion of these defects among the Russian Black-and-White population was 4.11, 2.96, 0.99, 2.88, 1.14, 2.23, 5.66, 1.00, 0.94 and 0 %, respectively. The frequency of the mutant allele was 2.05, 1.48, 0.49, 1.44, 0.57, 1.12, 2.83, 0.50, 0.47 and 0%, respectively for BY, HH1, HH2, HH3, HH4, HH5, HCD, CVM, BLAD and DUMPS (**Table 5**).

The proportion of carriers among bulls was 3.97, 1.71, 1.14, 3.83, 1.36, 2.36, 6.83, 0.57, 0.16 and 0% for BY, HH1, HH2, HH3, HH4, HH5, HCD, CVM, BLAD and DUMPS, respectively, while it was 4.52, 6.39, 0.60, 1.48, 0.52, 1.23, 2.61, 2.10, 2.94 and 0%, respectively among cows. The frequency of the mutant allele among bulls was 1.98, 0.85, 0.57, 1.69, 0.68, 1.31, 3.41, 0.29, 0.08 and 0% for BY, HH1, HH2, HH3, HH4, HH5, HCD, CVM, BLAD and DUMPS, respectively, while it was 2.26, 3.19, 0.30, 0.74, 0.26, 0.62, 1.30, 1.05, 1.47 and 0% respectively among cows.

49 animals have been detected as carriers for two different haplotypes. No carriers for more than two defects were identified. From the 78 animal that have been identified to be carriers for BY, 5 were carriers for HH1, 2 for BLAD, and 1 for each of HH3 and HH5. The carriers for Holstein haplotype HH4 have not been met to be carriers for other lethal haplotypes. Despite the large number of HCD carriers (94 animals), only 6 of them were carriers for another haplotype. The situations in which animals were identified as carriers of two different haplotypes is demonstrated in **Table 6**.

Table 5. Total carriers, proportion of carriers and the frequency of mutant alleles for the different studied haplotypes among the Russian Black-and-White cattle

	BY	HH1	HH2	HH3	HH4	HH5	HCD	CVM	BLAD	DUMPS
Total tested	1898	1521	1215	1529	1489	1433	1661	1697	1698	1617
Total carriers	78	45	12	44	17	32	94	17	16	0
Male carriers	56	19	10	38	15	27	82	7	2	0
Female carriers	22	26	2	6	2	5	12	10	14	0
Proportion of carriers in the population (%)	4.11	2.96	0.99	2.88	1.14	2.23	5.66	1.00	0.94	0.00
Frequency of the mutant allele in the population (%)	2.05	1.48	0.49	1.44	0.57	1.12	2.83	0.50	0.47	0.00
Proportion of carriers among bulls (%)	3.97	1.71	1.14	3.38	1.36	2.63	6.83	0.57	0.16	0.00
Frequency of mutant allele among bulls (%)	1.98	0.85	0.57	1.69	0.68	1.31	3.41	0.29	0.08	0.00
Proportion of carriers among cows (%)	4.52	6.39	0.60	1.48	0.52	1.23	2.61	2.10	2.94	0.00
Frequency of mutant among cows (%)	2.26	3.19	0.30	0.74	0.26	0.62	1.30	1.05	1.47	0.00

Table 6. The incidence of carriers of two different haplotypes among the studied animals of the Russian Black-and-White cattle

	BY	HH1	HH2	HH3	HH4	HH5	HCD	CVM	BLAD	DUMPS
BY	78	5	-	1	-	1	-	-	2	-
HH1	5	45	1	-	-	-	1	1	1	-
HH2	-	1	12	-	-	-	1	1	-	-
HH3	1	-	-	44	-	3	2	2	-	-
HH4	-	-	-	-	17	-	-	-	-	-
HH5	1	-	-	3	-	32	-	-	-	-
HCD	-	1	1	2	-	-	94	2	-	-
CVM	-	1	1	2	-	-	2	17	1	-
BLAD	2	1	-	-	-	-	-	1	16	-

DISCUSSION

The incidence of inherited anomalies in the cattle population has been reported in many countries such as: Australia (Windsor and Agerholm, 2009), China (Fang et al. 2013), Turkey (Oner et al. 2010), Brazil (Paiva et al. 2013) and Poland (Rusc et al. 2013). Our results clearly show the presence of these defects among the Russian Black-and-white cattle. The carrier and allele frequency of BY were relatively high 4.11% and 2.05%, respectively. Other high values of carrier frequency for BY were also reported from other countries: Belarus (3.38 %) (Mikhailova et al. 2018), China (4.9%) in bulls and 2.2% in cows (Fang et al. 2013) and the US (6.0%) (VanRaden et al. 2011). The proportion of carriers for the Holstein haplotype HH1 was 2.96%, and the frequency of the mutant allele was 1.48 %. This frequency is higher than the frequency reported in the German Holstein (0.88%) (Segelke et al. 2016). In the US Holsteins, the carrier frequency of HH1 exceeded 8% between the 1980s and 1990s, However, selection against this haplotype has reduced the frequency to 2% in 2015 (Adams et al. 2016). The carrier frequency for HH2 was 0.99%. This is much lower than the frequency of HH2 (4.6%) reported by VanRaden et al. (2011) in the US Holsteins. The allele frequency for HH2 (0.49%) is approximately twice lower than the allele frequency in the German Holstein (0.94%) (Segelke et al. 2016; Gebremeskel, et al. 2016).

According to our results, the value of carrier frequency for HH3 in the Russian Black-and-white cattle (2.88%) was approximately similar to those reported in the US – 2.95% (Cole et al. 2016), France – 3.1% (Fritz et al. 2018) and Germany – 3.29% (Segelke et al. 2016). Similar situation is observed for the HH5 with a carrier frequency of 2.23, 2.22, 2.76 and 1.9% in Russia, the US, Germany and France, respectively. However, the situation is a little different for HH4. The carrier frequency for this haplotype in the Russian Black- and-white cattle (1.14%) is higher than the frequency in the US Holsteins (0.37 %) (Cole et al. 2016), but lower than the frequency in the French Holsteins (4.4%) (Fritz et al. 2018).

A high value of carrier frequency of HCD was detected in this study (7.12%). Similar results for HCD was also reported in German Holstein Friesian with a carrier frequency of 6.7% (Schütz et al. 2016). However, lower frequency of HCD carriers was reported among the US Holsteins (2.5%) (Cole et al. 2016).

The Complex vertebral malformation (CVM) and Bovine leukocyte adhesion deficiency (BLAD) are considered the two most frequent inherited diseases occurring in Holstein cattle (Zhang et al. 2012). However, according to our results, the prevalence of these defects among the Russian Black-and-white cattle was moderate with a carrier frequency of about 1.2% for each. This value is lower than the values recorded in other countries. In Turkey, for example, the carrier frequency was 4.0% and 3.4% for BLAD and CVM, respectively (Meydan et al. 2010). A high value of carrier frequency for CVM was reported in Poland (16.62%) (Rusc et al. 2013), Sweden (23 %.) (Berglund et al. 2004) and Australia (4.44%) (Windsor and Agerholm,

2009). The prevalence of BLAD was also high in countries such as Germany (24%) (Zhang et al. 2012) and Brazil (5.7%) (Ribeiro et al. 2000), while it was 2.8% among the Iranian Holstein (Rahimi et al. 2006).

Our results indicate the absence of DUMPS among the Russian Black-and-white cattle. There were no positive result for this defect in any of the tested animals. The absence of DUMPS was also reported in Turkey (Kaya et al. 2016), the Czech Republic (Citek et al. 2006), India (Patel et al. 2006) and Brazil (Paiva et al. 2013). According to Kaya et al. (2016), the frequency of the mutant allele of DUMPS is relatively low or not exist in many parts of the world. However, Robinson et al. (1993) has reported the identification of 585 DUMPS carriers out of 3461 animals were tested in North America between the years 1988 and 1991, and 414 carriers out of 1226 animals were tested in Europe at the same period.

Identification of the animals that are carriers of genetic disorders based on the phenotypic reports is difficult due to phenotypic resemblance to normal animals. Only the genomic testing can reveal the carriers of mutations. Breeders should test their animals before using them in the mating program. Once carriers of genetic defects were identified, they should be mated to non-carriers to avoid further dissemination of the mutation, and to avoid the economic loss in their herds.

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ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

All experiments followed the instructions for the use of animals as experimental subjects of the Russian Federation.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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