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Short communication

Screening of haplotype for cholesterol deficiency genetic defect in the Russian Holstein cattle population

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Abstract

In our study, we estimated the frequency of haplotype for cholesterol deficiency (HCD) carriers in the Russian Holstein cattle population. We studied 1817 random samples of cows born in 2010-2017 from ten herds and 331 cows and heifers from the other three herds born in 2016-2019, fathers or fathers of mothers of which were HCD carriers. The method of AS-PCR was used for animals genotyping. In the first group of animals, the incidence of HCD carriers was 8.09%, and in the second one - 23.26%. Our results demonstrated the necessity to test cows for the carriage of the HCD genetic defect in the Russian population of Holstein cattle.

Key words: cows, Holstein breed, genetic defect, cholesterol deficiency

Introduction

Haplotype cholesterol deficiency (HCD) is a genetic defect in Holstein cattle that leads to the death of calves in the early postnatal period. It was first registered in 2015 in Germany. Pedigree analysis identified that the founder of the defective haplotype was Maughlin Storm bull, born in 1991 (Kipp et al. 2015). Using genome-wide sequencing, it was determined that this mutation is the insertion of 1233 bp long in the fifth exon of the *APOB* gene (apolipoprotein B) located in BTA 11, which leads to a shift in the reading frame and protein truncation (Menzi et al. 2016).

The aim of the study was monitoring of the incidence of the HCD genetic defect in the Russian population of Holstein cattle.

Materials and Methods

All experiments were carried out in accordance with the principles of the Helsinki Declaration (World

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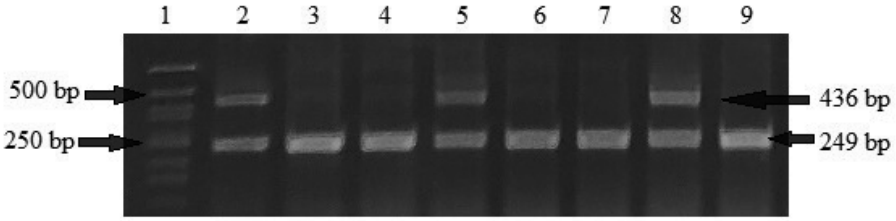

Fig. 1. Identification of heterozygous haplotype for cholesterol deficiency (HCD) carriers among cows. Line 1 - 50bp DNA ladder marker. Lines 2, 5 and 8 are heterozygous HCD carriers (two fragments were amplified - 436bp and 249bp). Lines 3, 4, 6, 7, and 9 - normal animals (one fragment of 249bp was amplified).

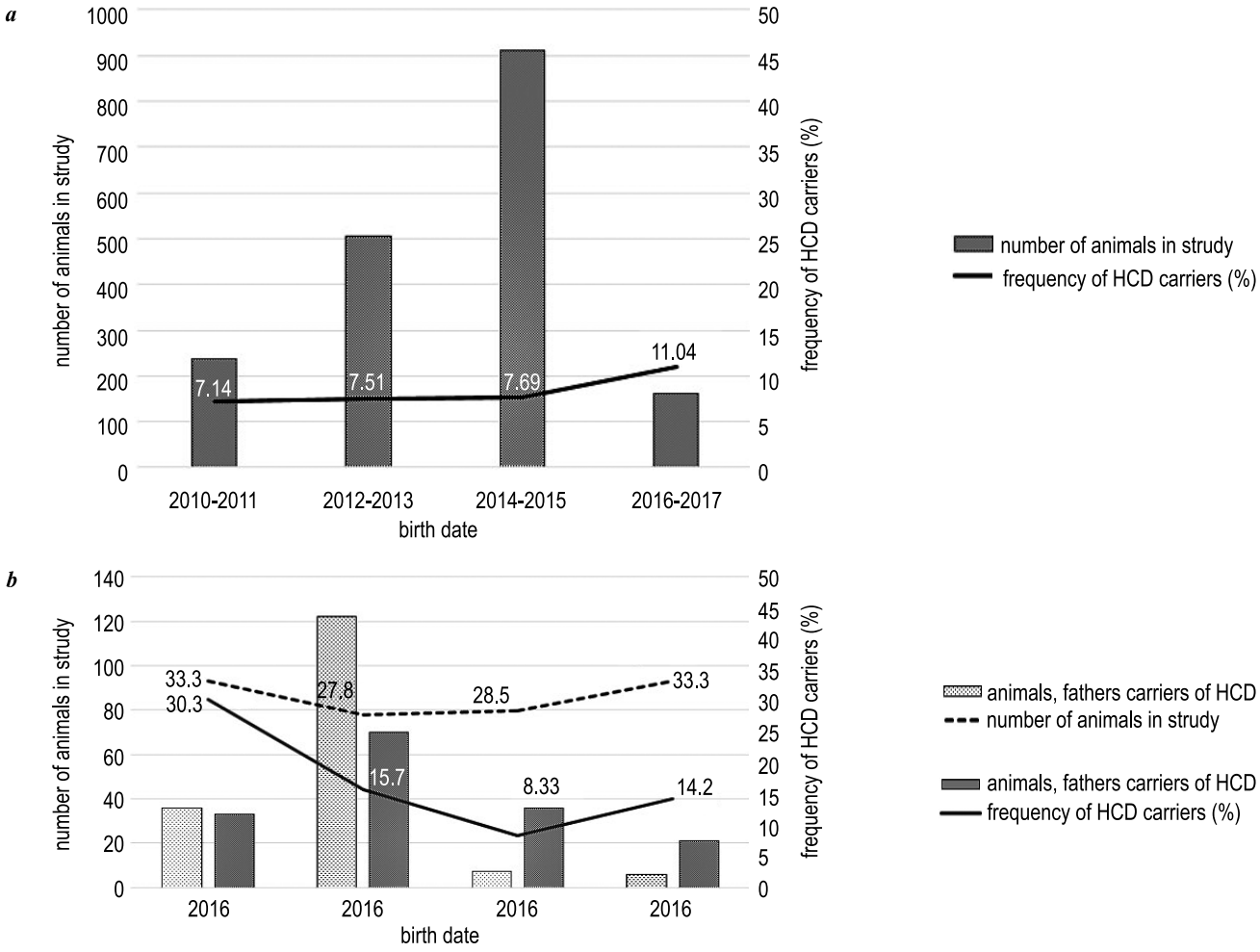

Fig. 2. HCD frequency in the Russian Holstein cattle population; a) - HCD frequency in a random sample of cows born in 2010-2017; b) - frequency of HCD in the sample of cows and heifers born in 2016-2019 in the pedigrees of which fathers or mother's fathers were HCD heterozygous carriers.

Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects, 1964-2013). We studied 1817 cows born in 2010-2017 belonging to ten farms. The cow selection was random. Another sample of 331cows and heifers born in 2016-2019 was also selected, in which the fathers (n=171) or mother's fathers (n=160) were heterozygous carriers of HCD. These animals were not included in the first sample and belonged to three other farms.

Blood samples were taken from the tail vein. Genomic DNA was isolated from blood samples using the phenol/chloroform extraction method. DNA concentration ranged from 50 to 500 ng/µl. All the animals were investigated according to the method proposed by Kamiński and Ruść (2016). PCR was performed in 10 µl of the reaction mixture containing 67 mM Tris-HCl, pH 8.6, 2.5mM $MgCl_2$, 16.6mM NH_4OH, 0.125mm each of deoxyribonucleotide triphosphates (dATP, dGTP, dCTP, dTTP), 0.5 µM

primer, 50-100 ng of genomic DNA and 2.5 units of Taq polymerase (Sibenzim LLC, Novosibirsk). The reaction was carried out on a Thermal Cycler T100 (Bio-Rad, USA) according to the following protocol: denaturation 94°C - 4 min, then 35 cycles, 94°C - 1 min, primer annealing - 62°C - 30 sec., elongation step 72°C - 1 min, the final synthesis step - 72°C 4 min. To identify the carriers of the recessive HCD mutation (indel polymorphism), the animals were genotyped using allele-specific primers: F1 5'GGTGACCATCCTCTCTCTCTGC3' - amplifies the 249bp fragment, F2 5'CACCTTCCGCTATTCGAGAG3' – amplifies 436 bp fragment where there is an insert of a mobile LTR element in the *APOB* gene, and a single reverse primer R5'AGTGGAACCCAGCTCCATTA3. For the separation of DNA fragments, the method of horizontal electrophoresis in 2% agarose gel containing 0.1 µg/ml of ethidium bromide at 10 V/cm in 0.5×TBE buffer for 40 minutes was used.

Results and Discussion

A total of 2148 animals were checked. Heterozygous HCD animals had two fragments sized 436bp and 249bp, while in the healthy animals only 249bp fragment was amplified (Fig. 1). In a sample of cows (n = 1817), 147 animal carriers of HCD (8.09%) were identified. The analysis showed that the maximum frequency of HCD in the analyzed sample of the cows was observed in the animals born in 2016-2017 (Fig. 2a). In the group of animals (n = 171), fathers of which were carriers of HCD, 50 cows had heterozygous HCD genotype (29.23%). The highest incidence of HCD mutation was observed among animals born in 2016 and 2019 (33.30%). Among cows and heifers (n = 160), the mothers of whose mothers were heterozygous carriers of HCD, 27 animals inherited this genetic defect (16.87%). A higher percentage of HCD carriers was determined in the animals born in 2016 (Fig. 2b). In general, 23.26% of the animals in the entire group were heterozygous carriers of the HCD genetic defect. A high incidence of the HCD genetic defect (12.5%) among bulls in Germany (Schütz et al. 2016) and China (5.07%) (Li et al. 2018) were reported. Given the high percentage of HCD carriers in the Holstein breed, this genetic defect is included in the genetic testing of bulls. To reduce economic losses and prevent the birth of homozygous animals, it is also necessary to test cows and young heifers.

Acknowledgements

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