Short communication: Distribution of recessive genetic defect carriers in Chinese Holstein

D. X. Sun,* X. H. Fan,* Y. Xie,* Q. Chu,* Y. Sun,* Y. Zhang,* S. L. Zhang,* W. J. Gong,† S. H. Chen,† Y. H. Li,‡
W. H. Shi,‡ and Y. Zhang*¹

*College of Animal Science and Technology, Key Laboratory of Animal Genetics and Breeding of Ministry of Agriculture, National Engineering Laboratory for Animal Breeding, China Agricultural University, Beijing 100193, China

†Dairy Data Processing Center, Dairy Association of China, Beijing 100085, China

‡Beijing Dairy Cattle Center, Beijing 100085, China

ABSTRACT

In dairy cattle, 4 important recessive hereditary diseases exist: complex vertebral malformation (CVM), bovine leukocyte adhesion deficiency (BLAD), citrullinemia (CTLN), and deficiency of uridine monophosphate synthase (DUMPS). Holstein Associations in developed countries have established monitoring systems for such disorders in Holstein bulls for decades. Over the past decades, China has continuously imported Holstein semen and embryos, mainly from North America but also from Europe. The dissemination of such genetic defects was undetermined until now, although efforts were taken to develop molecular techniques and detect carriers for CVM and BLAD in small populations of Chinese dairy cattle. Thus, herein we extensively screened 732 proven bulls participating in artificial insemination programs and 136 young bulls entering progeny test from 15 bull stations in China for CVM, BLAD, CTLN, and DUMPS. The proportion of carriers of the defects was found to be 7.72, 1.38, 0.23, and 0.12%, respectively. Given our findings, early diagnostic and monitoring systems on recessive inherited disorders among proven and young bulls entering the national genetic improvement programs for dairy cattle of China should be established immediately, in which a series of measures will be taken to prevent further spreading of such disorders and gradually eliminate them in the dairy cattle population in China.

Key words: complex vertebral malformation (CVM), bovine leukocyte adhesion deficiency (BLAD), citrullinemia (CTLN), deficiency of uridine monophosphate synthase (DUMPS)

Short Communication

Genetic disorders are hereditary physical or functional abnormalities with a negative effect on vitality. In animals, an important effect is a decrease in or loss of performance (DGfZ, 1986). In Holstein cattle, 4 important autosomal recessive diseases exist: complex vertebral malformation (CVM), bovine leukocyte adhesion deficiency (BLAD), citrullinemia (CTLN), and deficiency of uridine monophosphate synthase (DUMPS). Complex vertebral malformation results in decreased fertility, aborted fetuses, and stillborn calves. Calves affected with CVM who survive until late gestation exhibit a combination of symptoms with malformed legs and fused vertebrae (Agerholm et al., 2001; Duncan et al., 2001). Calves affected with BLAD suffer frequent and recurrent bacterial infections resulting in premature death (Kehrli et al., 1990). As for CTLN, a homozygous condition, the affected calves always die within 7 d of age because of urea cycle defects and induced higher content of ammonia in blood (Harper et al., 1986; Healy, 1996). Homozygous embryos for DUMPS do not survive to birth and usually appear to be aborted or reabsorbed at 40 to 60 d after gestation due to the complete loss of uridine monophosphate synthase activity (Shanks and Robinson, 1989; Shanks et al., 1992). These defects have brought severe economic losses to farmers and the dairy industry, and thus, their occurrence has been monitored and recorded for decades. Holstein associations in most developed countries have established efficient monitoring systems for recessive disorders for young and proven bulls, in which nearly all bulls with suspected pedigrees are tested for such defects. In such systems, whether a bull is a carrier for such diseases is recorded in its pedigree and stored in a common accessible database. Consequently, the proportion of CVM, BLAD, CTLN, and DUMPS carriers is very low in developed countries today. The 4 recessive defects are caused by single nucleotide mutations changing protein or enzyme amino acid sequences or shortening the protein (enzyme) size (Dennis et

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¹Corresponding author: changy@cau.edu.cn

al., 1989; Shuster et al., 1992; Schwenger et al., 1993; Thomsen et al., 2006). Accordingly, several PCR-based molecular techniques have been developed and are widely applied in such monitoring systems.

With the wide use of AI and international trading of semen and embryos, recessively inherited diseases have already been spread worldwide, especially through elite sires. It has been reported that Pennstate Ivanhoe Star was the common ancestor of bulls carrying CVM and BLAD and that these 2 defects were spread throughout the world mainly by his son, Carlin-M Ivanhoe Bell (Thomsen et al., 2006). In the case of DUMPS, most carriers across the world were descendants of Happy-Herd Beautician, a US Holstein bull born in 1987 (Robinson et al., 1993b). Another US Holstein sire, Gray View Crisscross, was responsible for CTLN; however, it was disseminated by his son, a Canada-born bull, Linnack Kriss King, whose semen was extensively used in Australia during the 1980s because his EBV for milk fat was excellent (Robinson et al., 1993a).

The Chinese Holstein originated from cross-breeding between the Chinese Yellow cattle and European Holstein over the past 100 yr, and foreign Holstein bulls, semen, and embryos, mainly from the United States, Canada, and Europe, have been imported for genetic improvement of Chinese Holstein (Sun et al., 2009). Considering this genetic background, it was presumed that major genetic defects would occur in the Chinese dairy cattle population. Nevertheless, their occurrence has never been extensively screened for service sires and young sires in China, although several molecular diagnostic techniques have been developed (Sun et al., 2007; Chu et al., 2008) as dairy cattle breeding in China is still evolving. Since 1983, the Dairy Association of China (DAC; Beijing, China) has organized a national dairy bull progeny test, and in total, 1,311 bulls from 44 batches have been tested through 2010 (a number much lower than those in developed countries). Thus, the primary purpose of this study was to thoroughly test all breeding bulls participating in the AI program and young bulls entering the national progeny test for CVM, BLAD, CTLN, and DUMPS to determine the distribution of the disorders and minimize the risk of spreading them among future bulls or bull dams in China.

Semen samples of 868 Holstein bulls consisting of 732 proven bulls and 136 progeny-test young bulls from 15 AI stations were provided by the Dairy Data Processing Center of DAC and tested for 4 genetic defects: CVM, BLAD, CTLN, and DUMPS. These proven bulls represented all active service sires participating in the national genetic improvement programs for dairy cattle in China from 2000 to 2010, including the majority of young bulls born after 2006. Genomic DNA was ex-

tracted from semen cells using a TIANamp Genomic DNA kit (TianGen, Beijing, China). Single strand conformation polymorphism (SSCP)-PCR (Chu et al., 2008) and RFLP-PCR (Sun et al., 2007) techniques were implemented to detect CVM and BLAD, respectively. With regard to CTLN and DUMPS, individual genotypes were defined with RFLP-PCR methods as described by Dennis et al. (1989) and Schwenger et al. (1993, 1994), respectively. To avoid false positive or false negative results, based on genotyping with aforementioned methods, we randomly selected 2 samples from each genotype of each disorder and then had their PCR products sequenced. The sequences of 2 random samples were found to be completely consistent with each other, indicating that our detection results were correct and reliable.

Through detection, 67 CVM carriers, 12 BLAD carriers, 2 CTLN carriers, and 1 DUMPS carrier out of 868 bulls were found, so that the proportion of the 4 recessive abnormalities was 7.72, 1.38, 0.23, and 0.12%, respectively. Thus, the frequencies of undesirable alleles corresponding to CVM, BLAD, CTLN, and DUMPS in the Chinese dairy cattle population were 3.86, 0.69, 0.12, and 0.06%, respectively. Then, pedigrees of all carrier bulls identified were traced back by using the online search procedures of DAC (<http://www.holstein.org.cn>), Holstein Association USA (<http://www.holsteinusa.com>), Holstein Canada (<http://www.holstein.ca>), and Holstein Australian (<http://www.holstein.com.au>). In accordance with expectation, 50 CVM carriers and 8 BLAD carriers were identified to be descendants of Carlin-M Ivanhoe Bell, of which 4 bulls were carriers of both CVM and BLAD. Coincidentally, 2 of the 4 CVM/BLAD carriers were twin-born from an imported embryo. On the other hand, as presumed, both CTLN carrier bulls were offspring of Linnack Kriss King. The only DUMPS carrier possessed blood of a US bull named Skokie Sensation Ned, who is the ancestor of Happy-Herd Beautician (Robinson et al., 1993b). During the past 10 yr, these carrier sires, especially the CVM and BLAD carriers, are the top sires in China and have been widely used throughout the country; many of their offspring are in the same herds. Based on the latest statistics released by DAC, about 340,000 Holstein cows are participating in the national DHI program, which accounts for about 5% of the whole dairy cattle population of China currently, of which about 50,000 cows are the daughters of the CVM, BLAD, CTLN, and DUMPS carrier sires detected in this study. Many suspected offspring of such heterozygous sires from small-scale farms are not included in the statistical data. Moreover, a monitoring system for the recessive genetic defects has not yet been established in China, and whether a young bull is a carrier of an undesirable

allele of inherited disorder is not considered when the animals enter the progeny test program. Hence, frequencies of the recessive defect gene for CVM, BLAD, CTLN, and DUMPS in Holstein cows would be close to that of bulls in China.

In addition, because many Holstein cows were imported from Australia in recent years, where the CTLN defect was first reported, 200 imported Australian cows from the Beijing region were tested for CTLN using the same diagnostic method as that for bulls in the present study. As a result, 5 cows were determined to be carriers of CTLN. Two of these heterozygous cows have complete pedigree information; one is a descendant of Linnack Kriss King, and the other is not.

The present study first extensively detected all AI and progeny-test Holstein bulls for CVM, BLAD, CTLN, and DUMPS (representing 4 of the most important and critical recessive inherited diseases in Holstein cattle) and determined their distribution profile. Our findings indicated that the current status of CVM carriers in China is similar to that in developed countries about 10 yr ago, in which systematically testing sires in AI for these defects has greatly reduced the frequency of heterozygous sires. It has been reported that 18% of US Holstein bulls marketed were CVM carriers in 2001, whereas less than 1% of the progeny test bulls born after 2002 are known carriers (VanRaden and Miller, 2006). In the German Holstein sire population, the frequency of carriers has decreased from 16.6% in 2002 to 4.6% in 2007 (CVM) and from 18.8% in 1997 to 0.6% in 2007 (BLAD; Schutz et al., 2008). Given such results, we believe that an early diagnostic and monitoring system for inherited defects for proven bulls and young bulls entering the national genetic improvement programs in China is urgently needed, for which the following necessary measures should be taken. First, CVM, BLAD, CTLN, or DUMPS carrier bulls should be identified as such in their official pedigrees. Second, because it is not economically beneficial to eliminate CVM and BLAD carrier bulls at one time, all suspected offspring of the bull carriers should be screened for these 2 defects. Furthermore, pedigrees of the cows should be examined to avoid using carrier bulls on carrier cows, especially when they are used in the planned mating program. For CTLN and DUMPS, it is more feasible to eliminate carrier bulls as they are revealed because of their low prevalence. Third, all young bulls should be tested before preselection for entering the progeny test program to avoid further spread of defective alleles to a larger population. Through these measures, it will be possible to prevent the occurrence of affected calves and reduce the negative influence on breeding. The proportions of

CVM, BLAD, CTLN, and DUMPS carriers will gradually decrease in Chinese dairy cattle.

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