A Retrospective Survey of the Prevalence of Complex Vertebral Malformation Carriers in 9 Holstein Dairy Herds in Hokkaido, Japan

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ABSTRACT. The carrier rates of Complex Vertebral Malformation (CVM) in 9 Holstein dairy herds in Hokkaido, number of usages of CVM carrier semen for breeding and gene frequencies of CVM carriers were measured. The mean CVM carrier rates of 140 cows from 4 herds in 1994 and 315 cows from 5 herds in 2003 were 10.8%(range 4.7–30.0%) and 5.1%(range 0.0–6.1%), respectively. The rate of use of CVM carrier semen in the Hokkaido district was 5.6% in 2002. The gene frequencies calculated from CVM carriers among the 315 cows and number of CVM carrier semen samples used were 0.032 and 0.028, and the occurrence of homozygous CVM in 2003 was estimated to be 0.1% in the local districts of Hokkaido, Japan.

NOTE. Theriogenology

Carriers in 9 Holstein Dairy Herds in Hokkaido, Japan

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Complex vertebral malformation (CVM) is an autosomal recessive inherited disorder in the Holstein breed, leading to death in uterus, abortion of fetuses and vertebral anomalies [1]. It is caused by a mutation in the Golgi-resident nucleotide-sugar transporter of a uridine diphosphate -N acetylglucosamine encoded by SLC35A3 [15]. In stillborn and aborted calves, CVM is characterized by shortened cervical and thoracic regions of the vertebral column, bilateral symmetric contraction of the metacarpophalangeal and metatarsophalangeal joints and symmetric arthrogryposis [1, 5, 10]. The occurrence of CVM in Holstein calves has been reported in European countries [1, 3, 13, 16], the United States [5] and Asia [10]. Extensive use of several sires of the Holstein breed that were CVM carriers is responsible for the widespread propagation of CVM carriers [15]. Since discovery of this defect and initiation of DNA testing in 2001, bulls determined to be carriers of this genetic disorder have been designated by the letters “CV”, and bulls that are not carriers have been listed as “TV” for artificial insemination reference purposes. Holstein bulls in Japan have been screened, and a list of “CV” bulls has been published [12]. However, the CVM situation in Japanese dairy herds has not been fully studied, and little data are available about the frequency of CVM carriers in Holstein populations. This paper reports the results of prevalence and allele frequency estimation of CVM in dairy cattle in Hokkaido, Japan, in the years 1994 and 2003.

Blood samples collected from 140 Holstein dairy cows from 4 local herds in which calves affected with bovine leukocyte adhesion deficiency (BLAD) were detected in 1994 [9] and used to measure the CVM carrier prevalence. Blood samples were also collected in 2003 from 315 Holstein dairy cows from 5 dairy herds, including 1 CVM-affected calf that was detected on 1 dairy farm [10], and used to measure the CVM carrier prevalence. Ten ml of blood was collected from the tail vein of the animal into a tube containing heparin and was used for DNA-polymerase chain reaction (PCR) analysis.

Pedigree analysis of the sires of CVM-carriers was performed using their registration certificates [12]. The number of semen specimens from CVM carriers used for artificial insemination was estimated as the total number of semen specimens used in the district of Hokkaido, Japan, in 2002 [4]. The number of bulls used for artificial insemination was determined from the list of the Top 40 Nippon Total Profit Index (NTP best 40) in 2002 [12].

Detection of the CVM-related gene was performed by DNA-PCR analysis as described previously [6]. The results were judged as normal, carrier or homozygous for CVM based on the pattern of the amplified products after gel electrophoresis.

The gene frequencies of CVM-free cattle and CVM carriers were calculated based on the Hardy-Weinberg law [17]. Estimation of the occurrence of CVM-affected cattle in Holstein herds was calculated based on the gene frequencies of CVM carriers. The differences in CVM carrier prevalence among the 2 groups of dairy herds were analyzed by $\chi^2$ analysis, and values of $p<0.05$ were regarded as significant.

In analysis of DNA from samples collected from the dairy herds, the CVM carrier prevalence of the 140 dairy cows from the 4 local dairy herds in 1994 ranged from 2.9 to 30.0%, and the mean CVM carrier prevalence was 10.8% (Table 1). This finding indicated that the CVM-related gene was widespread in Holstein dairy herds before the presence of CVM was confirmed in Holsteins. In 2003, the CVM carrier prevalence of the 315 dairy cattle from the 5 dairy herds...
herds in 2003 ranged from 0.0 to 8.1%, and the mean CVM carrier prevalence was 5.1%. The decreased rate of CVM carriers in the dairy herds in 2003 compared with the rate in 1993 may be associated with elimination of bulls that were BLAD carriers and CVM carriers. However, the mean carrier rates for 1994 and 2003 were not significantly different. This suggests that a few bulls that were CVM carriers were used continuously for breeding of dairy cattle and that consequently CVM carriers became widespread. The ages of CVM carriers ranged from 1 to 9 years in 2003. The highest gene frequency for CVM was 0.150, which was found in a herd in which the CVM carrier prevalence was 30% (9/30) in 1994. Although the number of blood samples collected from the dairy herds that were tested with DNA-PCR was small and the regions where blood was collected from dairy cattle were limited, the prevalence of CVM carriers in Holstein cattle in the local districts of Hokkaido can be estimated to range from 5.1 to 10.8% based on the values for 1994 and 2003.

In a pedigree analysis, the elite bulls Carlin-M Ivanhoe Bell (BLAD/CVM carrier), Penstate Ivanhoe Star (BLAD/ CVM carrier) and Osbondale Ivanhoe (BLAD carrier) were genetically linked, and it has been suggested that the origin of CVM is either Penstate Ivanhoe Star, the sire of Carlin-M Ivanhoe Bell, or from the maternal side [15]. Carlin-M Ivanhoe Bell was also a carrier of the genetic disorder BLAD [15], which is a defect caused by a recessive mutation in the gene encoding leukocyte $\beta_2$ integrin [14]. Elite bulls descending from Carlin-M Ivanhoe Bell have been used in dairy cattle breeding worldwide for 20–30 years. The mean rate of CVM carriers, 10.8%, in the 4 dairy herds in 1994 was similar to the rate of BLAD carriers calculated from 20 dairy herds in 1994, 5.4%–10.8%, as reported previously [9]. Based on these findings, it appears that extensive use of Holstein sires that were heterozygous carriers of BLAD and CVM has increased the number of BLAD and CVM carriers in Holstein cattle over a long period of time.

In regard to studies of the effects of this defect on the dairy industry, Berglund et al. [3] evaluated how Holstein bulls heterozygous for CVM influence reproductive performance and, in a daughter fertility index, showed that CVM carriers have an inferior non-return rate, as evaluated by relative breeding values, compared with non-carriers. The presence of CVM carriers in the Holstein breed appears to have caused significant economic losses to the dairy industry for a considerable period of time [7, 8, 11].

The number of CVM carriers in a given herd is considered to be dependent on past usage of semen from carrier bulls. In Japan, 13 of the 40 sires (32.5%) listed in the NTP best 40 were identified as CVM carriers in 2002 [12]. As the occurrence of homozygous CVM in dairy farms depends on selective use of bulls that are CVM carriers, it is difficult to know the exact rates of usage of such bulls for artificial insemination in each dairy herd. In this study, the occurrence of homozygous CVM in dairy cattle was estimated based on the prevalence of cows that were CVM carriers and the number of semen specimens used for artificial insemination in Hokkaido [12]. The gene frequencies of CVM carriers among 315 dairy cattle and semen specimens from CVM-carrier bulls used for cattle breeding in the Hokkaido

### Table 1. Prevalence of CVM carriers in Holstein dairy cows from 9 local herds in the Hokkaido district in 1994 and 2003

<table>
<thead>
<tr>
<th>Year</th>
<th>Herd</th>
<th>No. of carriers/ No. of cows tested</th>
<th>Carriers (%)</th>
<th>Gene frequency of CVM carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>A</td>
<td>9/30</td>
<td>30.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1/34</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>3/55</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>1/21</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14/140a)</td>
<td></td>
<td>10.8b)</td>
<td>0.050</td>
</tr>
<tr>
<td>2003</td>
<td>E</td>
<td>3/49</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>4/71</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>3/51</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>0/20</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>10/124</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20/315a)</td>
<td></td>
<td>5.1b)</td>
<td>0.032</td>
</tr>
</tbody>
</table>

a) Total number of carriers/total number of cows tested.

b) Mean of carrier rate (%).

### Table 2. CVM carriers and gene frequency in semen specimens used for cattle breeding in the local districts of Hokkaido

<table>
<thead>
<tr>
<th>Units of semen useda)</th>
<th>Normal carriers</th>
<th>CVM carriers</th>
<th>Total carriers</th>
<th>Carriers prevalence(%)</th>
<th>Gene frequency of CVM carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>693204</td>
<td>40796</td>
<td>734000</td>
<td>5.6</td>
<td>0.028</td>
<td></td>
</tr>
</tbody>
</table>

district were 0.032 and 0.028, respectively (Table 2). Semen of elite bulls from among the top sires has been used in Japan, and use of this semen appears to be associated with the increase in carrier frequencies in dairy cattle, as shown in the rank of the NTP best 40 [12]. Based on the gene frequencies of CVM carriers among dairy cows and the number of semen specimens of CVM carrier bulls used, the occurrence of homologous CVM in Holstein cattle was estimated to be 0.1% in dairy herds in 2003 in the local districts of Hokkaido, Japan. This value has clearly decreased over time. Elimination of bulls carrying CVM from Holstein herds would be the most efficient method of controlling this genetic disorder; however, such bulls were still listed in 2007 as commercially available for artificial insemination due to their values as genetic resources for breeding.

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