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Original article

Sire pedigree error estimation and sire verification of the Taiwan dairy cattle population by using SNP markers

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Abstract

Information regarding the correct pedigree of and relationship between animals is useful for managing dairy breeding, reducing inbreeding, estimating breeding value, and establishing correct breeding programs. Additionally, the successful implementation of progeny testing is crucial for improving the genetics of dairy cattle, which depends on the availability of correct pedigree information. Incorrect pedigree information leads to bias in bull evaluation. In this study, Neogen GeneSeek Genomic Profiler (GGP) 50K SNP chips were used to identify and verify the sire of Taiwanese Holstein dairy cattle and analyze the reasons that lead to incorrect sire records. Samples were collected from 2,059 cows of 36 dairy farms, and the pedigree information was provided by breeders. The results of sire verification can be divided into three categories: submitted unconfirmed sire, submitted confirmed sire, and incorrectly submitted verified sire. Data on the sires of 1,323 (64.25%) and 572 (27.78%) dairy cows were verified and discovered, respectively. Sires of 1,895 (92.03%) dairy cattle were identified, which showed that the paternal pedigree of dairy cattle could be discovered and verified through genetic testing. An error-like analysis revealed that the data of 37 sires were incorrectly recorded because the bull's NAAB code number was incorrectly entered into the insemination records: for 19 sires, the wrong bull was recorded because the frozen semen of a bull placed in the wrong storage tank was used, 6 had no sire records, and for 12 sires, the NAAB code of the correct bull was recorded but with a wrong stud code, marketing code, or unique number for the stud or breed. To reduce recorded sire error rates by at least 27.78%, automated identification of the mated bull must be adopted to reduce human error and improve dairy breeding management on dairy farms.

Key words: Holstein cattle, genetic testing, sire pedigree

Introduction

Implementation of the progeny testing program is essential for improving the genetics of dairy cattle. Genetic evaluation can be used to assess the genetic merit of dairy cows. Well-designed animal models are available, which are used to analyze performance records and genetic relationships between individual animals. An important assumption of these models is that all genetic relationships are correct. Misidentification of animal parents may lead to errors in mate selection, reduce genetic gain, and ultimately lead to loss

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of profits. Statistical losses have been reported due to pedigree errors. A 10% pedigree error rate could increase the coefficient of inbreeding by 6%-13%, result in an 11%-15% reduction in breeding value trends, and cause a 2%-3% loss in selection response (Banos et al. 2001, Visscher et al. 2002) and a resulting bias downward in heritability estimates (Israel and Weller 2000). Different markers can be used to verify parentage. Initially, blood group information was used (Stormont 1967). Microsatellite markers have been commonly used in the past two decades (Davis and DeNise 1998), but recently, SNP markers are predominantly used for parentage verification (Heaton et al. 2002, Werner et al. 2004).

The BeadChip, which comprises many genetic SNP markers, power applications such as genome-wide selection, quantitative trait loci identification, and genetic merit evaluation. In addition to the aforementioned uses, genotyping enables genetic defect detection and parentage identification. We recently analyzed the carrier frequencies of the genetic defect brachyspina, bovine leukocyte adhesion deficiency, complex vertebral malformation, deficiency of uridine monophosphate synthase, and mule foot in Holstein cows in Taiwan, and we corrected contrasting cases of the brachyspina genotype (Chao et al. 2020, 2021). The International Society for Animal Genetics (ISAG) approved standards for genotyping laboratories to conduct parentage verification in 2012. The Neogen Genomics laboratory in the United States, a CDCB--certified and ICAR-accredited laboratory for parentage verification in cattle by using SNPs, provides a rapid and efficient parentage determination service. Their bovine GGP SNP chips, which include all commonly used USDA and ISAG parentage SNP markers, are useful for parentage analysis.

Various paternity error rates have been reported in cattle populations worldwide 2%-5% in Israel (Ron et al. 1996), 12% in The Netherlands (Bovenhuis and van Arendonk 1991), 5%-15% in Denmark (Christensen et al. 1982), 8%-20% in Ireland (Beechinor and Kelly 1987), and 4%-23% in Germany (Gelderman et al. 1986). To date, no estimate is available on sire identification of Taiwan dairy herds. The aim of this study was to quantify the error level in sire identification and investigate sire verification frequency to analyze the patrilineal error patterns in Holstein cattle in Taiwan.

Materials and Methods

Hair follicles or blood samples were collected from 2,059 random cows in 36 herds (9, 10, 15, 1 and 1 herds from Northern, Central, Southern, and Eastern Taiwan and Kinmen Islands, respectively). Neogen's patented hair and blood sample collection card was used to collect hair follicles and blood for DNA processing and archiving. The sampling method involved pulling out approximately 30 hair follicles from each cow and using a syringe to collect blood samples from the tail vein. Two to three drops of blood were allowed to drip onto the collection card. Then, the sample collection cards were processed at 75°C for 30 minutes. The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the Taiwan Livestock Research Institute (LRI-IACUC107-5, LRI-IACUC108-1, and LRI--IACUC109-1). The data, including the date of birth, sire and dam of the tested cow and sample collection card were mailed to the Neogen Genomics Lab in Lincoln, NE, United States, and the GGP bovine 50K SNP chips that use Illumina Infinium technology (Illumina 2017) were used for genotyping. The CDCB-certified laboratory at Neogen uses the Illumina Infinium XT genotyping assay platform (Illumina 2017). The recommended workflow is as follows: "On the first day, the sample DNA is amplified, enzymatically fragmented, precipitated and resuspended. Then during the overnight incubation, the sample is hybridized to the BeadChip, where DNA anneals to locus-specific 50-mer probes covalently linked to one of the Infinium bead types. The next day, the Infinium XT workflow continues with enzymatic base extension to confer allelic specificity, followed by fluorescent staining. The iScan System detects the fluorescence intensities of the beads, and the Illumina software automatically performs analysis and genotype detection." The ICAR guidelines for parentage verification and parentage discovery based on SNP genotypes are used as standards for parental identification (ICAR 2017).

Results

The sire verification and misidentification frequencies of the 36 herds of Holstein cows in Taiwan are presented in Table 1. Sire verification and discovery results can be divided into three categories: submitted unverified sire, submitted verified sire, and incorrectly submitted discovered sire. The submitted sires of 164 (7.97%) and 1,323 (64.25%) dairy cows were unverified and verified, respectively. The incorrectly submitted sires of 572 (27.78%) dairy cows were discovered. A total of 1,895 (92.03%) dairy cattle sires were identified. The incorrectly submitted sire discovery rate can be regarded as the recorded sire error rate. Recorded sire errors were found in 32 of 36 herds, with 1-176 tested cows in each herd having a recorded sire error and error frequencies of 4.35%-100%. The average recorded sire error frequency was 27.78%. An error-like

Table 1. Sire misidentification frequencies among 36 herds of Holstein cows in Taiwan.

Herd	No. of genotyped cattle (A)	Submitted unverified sire (B)	Submitted verified sire (C)	Incorrectly submitted discovered sire (D)	Frequency of sire identification (%) (C+D/A)	Recorded sire error rate (%) (D/A)
1	30	14	4	12	53.33	40.00
2	137	15	107	15	89.05	10.95
3	85	7	48	30	91.76	35.29
4	187	1	149	37	99.47	19.79
5	20	4	10	6	80.00	30.00
6	19	2	17	0	89.47	0.00
7	92	0	88	4	100.00	4.35
8	20	4	11	5	80.00	25.00
9	39	0	28	11	100.00	28.21
10	40	0	35	5	100.00	12.50
11	10	0	8	2	100.00	20.00
12	10	1	9	0	90.00	0.00
13	9	1	6	2	88.89	22.22
14	9	0	6	3	100.00	33.33
15	156	45	58	53	71.15	33.97
16	10	0	4	6	100.00	60.00
17	53	0	49	4	100.00	7.55
18	5	4	0	1	20.00	20.00
19	20	0	3	17	100.00	85.00
20	9	0	7	2	100.00	22.22
21	412	21	215	176	94.90	42.72
22	20	0	20	0	100.00	0.00
23	19	4	13	2	78.95	10.53
24	10	0	9	1	100.00	10.00
25	298	6	205	87	97.99	29.19
26	6	0	3	3	100.00	50.00
27	10	1	8	1	90.00	10.00
28	9	0	8	1	100.00	11.11
29	38	0	31	7	100.00	18.42
30	17	0	0	17	100.00	100.00
31	128	15	72	41	88.28	32.03
32	50	12	30	8	76.00	16.00
33	12	0	11	1	100.00	8.33
34	14	0	12	2	100.00	14.29
35	48	1	37	10	97.92	20.83
36	8	6	2	0	25.00	0.00
Total	2059	164	1323	572	92.03	27.78

Table 2. Some cases of recorded sire error for cows tested at a dairy farm due to NAAB code entry errors.

Cases	Cow ID	Birth date	Sire submitted (Identification number) (NAAB code number)	Genomic sire (Identification number) (NAAB code number)	Status of reported sire
1	7050106F105	04/02/2017	HOUSA000066024146 007HO10711	HOUSA000066636657 007HO10721	Discovered
2	106F116	08/22/2017	HOUSA000001858296 007HO01419	HOUSA000069981350 007HO11419	Discovered
3	106F102	03/13/2017	HOUSA000069169948 001HO11201	HOUSA000139761263 011HO11201	Discovered
4	105F316	08/29/2016	HOUSA000069679487 007HO11427	HOUSA000069990138 007HO11477	Discovered
5	103F021	11/13/2014	HOUSA000001872264 007HO01585	HOUSA000069701759 007HO11585	Discovered
6	106F107	05/01/2017	HOUSA000071588470 011HO11360	HOUSA000065496393 011HO10360	Discovered
7	7050107F831	10/12/2018	HOUSA000132308618 007HO07507	HOUSA000071813323 029HO17507	Discovered
8	7050107F834	11/03/2018	HOGBR000000654429 029HO17057	HOUSA000071813323 029HO17507	Discovered
9	7050107F838	11/25/2018	HOGBR000000654429 029HO17057	HOUSA000071813323 029HO17507	Discovered
10	7050107F827	10/09/2018	HOBRA0000AX142531 029HO17928	HO840003127334847 029HO17918	Discovered
11	705000190916	10/08/2019	HO840003008328793 007HO11601	HO840003008897582 007HO11621	Discovered
12	705000190914	09/16/2019	HOUSA000071336919 029HO16775	HOUSA000070801821 029HO16575	Discovered

analysis of the 37 incorrectly submitted sires' record was conducted on our own dairy farm. Six cases had no bull record, 19 cases were recorded as other bulls because the other frozen semen samples in the same storage tank were recorded, and 12 cases were noted due to an attempt to mark the correct NAAB code number but with the wrong stud code, marketing code, or unique number of the stud or breed (Table 2).

Discussion

The BeadArray SNP genotyping platform (Illumina, CA, USA) used in this study is a high throughput and allele identification method (Oliphant et al. 2002, Kim and Misra 2007). Igenity parent verification is a highly accurate and cost-effective parentage identification tool. It includes all commonly used USDA and ISAG parentage SNPs. A total of 1,895 (92.03%) dairy cattle sires were identified. The identification showed that the paternal pedigree of dairy cattle could be discovered and verified through genetic testing. The dam was not genetically tested, but in cases where the imported frozen semen came from United States or Canada, most bulls had been genetically tested. If the dam is also subjected to genetic testing, the identification rate will improve.

The recorded sire error rate is based on the discovery rate of incorrectly submitted sires, although the "submitted unverified sire" section may also contain recorded sire errors. The average recorded sire error frequency was 27.78%. Our results showed a relatively large recorded sire error rate, which was higher than that in previous reports. Christensen et al. (1982) noted many reasons for such errors. We further analyzed the cause of the high recorded sire error rate. In many countries, the NAAB code is preferred to record mated bulls, which can cause errors. Thirty-seven incorrectly submitted sire records were conducted on our own dairy farm at which the bull's NAAB code naab number was entered into the insemination record. The 19 cases were wrongly recorded because the semen from other bulls was stored in the same storage tank. The main reason

could be that the AI technician misidentified the semen straw or entered the wrong bull's number into the insemination record. Furthermore, six cases did not even have bull records. The errors in another 12 cases were due to an attempt to mark the NAAB code but with the stud code, marketing code, or unique number of the stud or breed incorrectly inserted. Handwritten numbers can be easily misidentified. For example, a bull's NAAB code number 007HO10711 can easily be written as 007HO10721, 007HO01419 can easily be written as 007HO11419, and 001HO11201 can easily be written as 011HO11201. The stud, marketing code, and unique number of the stud and breed can easily be written incorrectly. Furthermore, analyzing the causes of such errors indirectly verified the correctness of the sire discovery and verification provided by the genetic testing service. Although some farms recorded sire pedigrees well, ample room for improvement exists, although most of cattle herds in Taiwan belong to commercial farms. The commercial farm must pay attention to herd pedigree accuracy. In addition to educating breeders to complete pedigree records, equipment must be introduced to accurately and conveniently identify cattle to prevent inaccurate records due to mistakes in handwriting or misreading as well as data input errors.

Such commercial genetic testing services provided by CDCB-certified and ICAR-accredited laboratories can be used as a screening model in many countries that do not regularly monitor paternal pedigree errors or have not yet conducted such surveys. This model is equivalent to having a professional laboratory assist in achieving breeding goals, and the analysis report is similar to that provided by DHI testing laboratories, facilitating optimal herd management.

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