GENETICS, BREEDING, AND MODELING

Relationship of Bovine Leukocyte Adhesion Deficiency with Genetic Merit for Performance Traits

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ABSTRACT

Examination of the existence of pleiotropy or linkage of bovine leukocyte adhesion deficiency with other traits and of the impact of removal of the recessive, undesirable allele on genetic progress for those traits has been limited. Frequency of carriers among 6400 Holstein bulls tested was 8.2%; however, reporting was incomplete, and, therefore, the estimate of carrier frequency was biased downward. For AI-sampled bulls, carrier frequency reached a high of 23% for bulls sampled during 1989 but declined to 0% since then because of DNA testing and culling. Association of the allele with yield, productive life, and somatic cell score was examined with a model in which the daughter yield deviation minus the mean of parent evaluations was explained by carrier status. A significant negative relationship was found with protein yield when effect of sires was ignored; all associations were unfavorable. Linkage was examined by applying the model for each of four sire families; only protein yield for one sire was significantly and negatively related to the recessive allele. Carrier bulls currently are labeled, and some continue to be used actively in AI because of superiority for other traits. Consequential pleiotropy of the allele or linkage of the locus with the traits studied is unlikely. Genetic progress for these performance traits will not be impeded by failure to sample carrier bulls.

(**Key words**: bovine leukocyte adhesion deficiency, DNA testing, linkage, pleiotropy)

Abbreviation key: **BLAD** = bovine leukocyte adhesion deficiency.

INTRODUCTION

A number of genetic recessive traits of Holstein cattle have been identified, and carriers are labeled when known. The Holstein Type-Production Sire Summaries (5) list nine such conditions, most of them undesirable and many fatal in the homozygous condition. Bovine leukocyte adhesion deficiency (**BLAD**), identified in 1989 (6), results in death of the homozygous calf because of its inability to defend against common infections. In affected calves, leukocytes lack a family of proteins, β_2-integrins, that are needed for mobilized leukocytes to pass through blood vessels to fight infection (6). Affected calves have been able to survive in poor condition to 3 yr only under intensive medical care (1); survival to maturity is theoretically possible only under sterile conditions.

Lack of other obvious manifestations caused these genetic disorders to go unidentified for many years until DNA testing revealed the presence of a point mutation in the CD18 gene for several generations. A formalin-fixed tissue sample contained the BLAD CD18 allele as far back as 1977 (3). Identified only for Holsteins thus far, BLAD carriers are among the most prominent bulls of the breed: Osborndale Ivanhoe, Penstate Ivanhoe Star, and Carlin-M Ivanhoe Bell (7). Osborndale Ivanhoe was born in 1952; therefore, the allele has existed for some time.

A test for the BLAD allele was available in 1991 (9). Late during that year, 109 of 815 bulls tested (13.3%) were found to be carriers (2). Of bulls in active AI service, 37 of 343 were carriers of the BLAD allele (2). Holstein bulls tested and reported to the Holstein Association USA, Inc. (Brattleboro, VT) had the test results appended to the bull name by January 1992. The codes are *BL for a BLAD carrier and *TL for a bull found to be free of the BLAD allele (2).

Although the gene frequency has been reduced dramatically among bulls that were progeny tested, the question has remained whether the prevalence of the BLAD allele was brought about by a positive association with yield or other traits that gave a heterozygous animal an advantage over homozygous normal animals. If so, elimination of carrier bulls would also result in a loss for performance traits. The purpose of this study was to document the frequency of BLAD carriers for the US Holstein bull population and to examine the relationship of the BLAD allele with genetic merit for yield, productive life, and somatic

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cell score. The existence of multiple effects of the BLAD allele (pleiotropy) and the location of the CD18 locus relative to genes that may be of major importance for performance traits (linkage) were examined to determine possible causes of any associations found between BLAD and the performance traits.

MATERIALS AND METHODS

Although the Holstein Association attaches suffixes to bull names to identify carriers or tested noncarriers of recessive alleles, some individual bulls carry more recessive alleles than can be reported in the allotted characters. Therefore, the Holstein Association provided a complete file of test results of bulls for BLAD to ensure the use of all results reported. This file was merged with USDA-DHIA performance files from January 1995; if available, genetic evaluations for milk, fat, and protein yields; fat and protein percentages; productive life; and somatic cell score were included. Frequencies of carriers were determined for sons of sires tested and found to be free of the BLAD allele, carriers, and untested sires by sampling years for AI-sampled bulls, which were defined as those with a sampling code of S to indicate that semen was distributed to at least 40 herds by an organization that would ultimately market bulls acceptable after sampling (8).

Dependent variables of milk, fat, and protein yields; fat and protein percentages; productive life; and somatic cell score were expressed as the difference between a bull mean for daughter yield deviation (10) and the mean evaluation for the bull parents. The fixed statistical model was daughter yield deviation – parent mean = allele status (BLAD allele present or absent) + error. To test the possibility of pleiotropy, the model was applied across all sons of carrier bulls in a daughter design (yield deviations were from daughters of subject bulls). Use of daughter yield deviation minus the parent mean allowed the effect of BLAD allele status to be examined relative to the expected merit from a random sample of parental genes. To examine possible linkage, the same model was applied separately for sons of widely used carrier sires in a granddaughter design (4). If a major gene or genes were located near the CD18 locus, association with the BLAD allele would be positive or negative depending on whether a favorable or unfavorable performance allele was on the same chromosome and closely linked to the normal or BLAD allele for that sire family.

RESULTS

Table 1 presents the frequency of carriers for data available in December 1994. Of the 6400 bulls tested for presence of the BLAD allele, 8.2% were carriers. Determining carrier (and allelic) frequencies was complicated because animals were not tested at random. For bulls with noncarrier sires, carrier prevalences of 3.3% among all bulls and 8.3% for sons evaluated for PTA for milk with ≥10 daughters indicated that allele frequencies in the female population would also be 3.3 and 8.3% and that carrier frequencies for females would be 6.6% for dams of all bulls and 16.6% for dams of evaluated bulls. The lower frequency for all bulls might indicate incomplete, selective reporting of BLAD status; for example, bulls that were found to be carriers might have been discarded and the condition unreported. Some missing data might have resulted from DNA testing prior to registration so that no identification number existed with which to match a report.

When the DNA test for determining the presence of the BLAD allele became available, perhaps 4000 to 5000 AI-sampled bulls were awaiting the results of progeny testing; many of those bulls had suspect pedigrees with regard to BLAD. As a cost-saving measure, only those bulls with favorable progeny-test results and that had been retained for future use in AI were tested for the BLAD allele. Other bulls were discarded before DNA testing, which resulted in a loss of data but not necessarily in a large bias in apparent carrier frequency. However, for bulls that were tested for the BLAD allele, results might have been reported more frequently for noncarriers than for carriers because carrier bulls were discarded and of no further interest. To promote more complete reporting in the future, free registration could be offered for animals that are disposed of so that a mechanism exists for

TABLE 1. Frequency of carriers of bovine leukocyte adhesion deficiency among Holstein bulls reported through December 1994.

	Bulls tested	Carriers among tested bulls
	(no.)	(%)
All bulls	6400	8.2
Noncarrier sire[1]	5163	3.3
Carrier sire[2]	683	44.7
Untested sire	554	9.6
Sons evaluated for PTA for milk with ≥10 daughters		
Noncarrier sire[1]	1776	8.3
Carrier sire[2]	598	49.3
Untested sire	512	9.8

[1]Registration designation of TL.

[2]Registration designation of BL.

TABLE 2. Numbers of Holstein bulls with results reported for DNA testing for the bovine leukocyte adhesion deficiency (BLAD) allele and carrier frequency by birth year of bull.

Birth year	Bulls tested for BLAD	Carrier bulls among tested bulls
	(no.)	(%)
1985	175	5.7
1986	374	9.9
1987	509	16.7
1988	669	24.2
1989	615	17.2
1990	789	5.2
1991	1043	0.3
1992	987	0.1
1993	789	0.3

data collection, statistics on prevalence can be improved, and dams can be identified as carriers when the sire is a noncarrier.

This hypothesis of incomplete reporting is supported by the carrier frequency of 44.7% for sons of sires that were carriers. If males were the only source of the allele, a carrier frequency of 50% would be expected for sons. A carrier frequency of >0% for females would result in a carrier frequency of >50% for sons of carrier bulls. For evaluated bulls with ≥10 daughters, carrier frequency for sons of carriers was nearly 50%, which was lower than expected because carrier frequency for dams certainly is >0%.

Carrier frequency for all bulls with results reported from a DNA test for the BLAD allele decreased from 24.2% for bulls born during 1988 to 0.3% for bulls born during 1993 (Table 2). Data for bulls sampled through AI (sampling code of S) are in Table 3 by year of entry into AI sampling. The number of bulls sampled during 1986 was low because the coding program was new and coding was incomplete. Before its discovery, the BLAD allele had reached a relatively high frequency: 23% of AI bulls sampled during 1989 that had reported tests for BLAD were carriers. Of those carriers, 74% were sons of 3 bulls. Even if all bulls without reported tests for BLAD during 1989 had been free of the allele, 11% of the bulls sampled would have been carriers. Of bulls sampled in AI during 1993 and 1994, 68% were tested for BLAD, and no carriers were found among them. This decrease in the frequency of BLAD carriers reflects AI sampling policy rather than a dramatic decline in allele frequency for the general population. The AI organizations implemented procedures that eliminated the progeny testing of BLAD carriers.

The 32% of bulls sampled in AI during 1993 and 1994 but not reported as tested likely were not carriers (J. R. Thompson, 1995, personal communication). Those bulls probably were not reported as tested because 1) testing was prior to registration and the results were not reported, 2) testing was done by a research laboratory or other organization that does not report results, or 3) both parents were noncarriers. Incomplete reporting of BLAD testing generally was unrelated to bull merit for the other traits examined and likely did not affect conclusions from statistical analyses. However, a tendency to report carrier status for bulls with high merit for desired traits could result in favorable solutions for carriers.

Least squares solutions for the effect of the BLAD allele on daughter performance for milk, fat, and protein yields; productive life; and somatic cell score (all adjusted for mean of parent evaluations) showed that carrier bulls were generally less desirable for performance traits (−18 kg of milk, −0.4 kg of fat, −0.9 kg of protein, +0.003 for fat percentage, −0.001 for protein percentage, −0.01 mo of productive life, and +0.001 for somatic cell score) but significant (P = 0.02) only for protein yield (Table 4). Any pleiotropic effect would probably be inconsequential.

Four carrier sires had >10 sons that were tested for BLAD and had USDA performance evaluations: Carlin-M Ivanhoe Bell*BL (Bell); 2 Bell sons, Lekker Ivanho Bell Jesse-ET*BL (Jesse) and Ripvalley NA Bell Troy-ET*BL (Bell Troy); and Thonyma Secret*BL (Secret). Bell is the son of Penstate Ivanhoe Star, who is the son of Osborndale Ivanhoe; all 3 bulls are BLAD carriers. The sire and maternal grandsire of Secret were not tested for the BLAD allele, and his paternal grandsire is a noncarrier. The sire of the maternal granddam of Secret is Penstate Ivanhoe Star, and the sire of his paternal granddam is Osborndale Ivanhoe; therefore, either great-grandsire likely was the source of the BLAD allele.

TABLE 3. Numbers of Holstein bulls entering AI sampling, percentages tested for presence of the bovine leukocyte adhesion deficiency allele, and percentages of tested bulls that were carriers by year of AI sampling.

Sampling year	Bulls sampled	Tested bulls among sampled bulls	Carrier bulls among tested bulls
	(no.)	(%)	
1986	555	22	5.0
1987	1004	24	10.7
1988	1134	38	13.1
1989	1100	48	23.0
1990	1201	47	19.2
1991	1227	40	8.5
1992	1284	77	0.4
1993	1193	69	0.0
1994	716	66	0.0

TABLE 4. Least squares solutions for effect of the allele for bovine leukocyte adhesion deficiency on daughter performance[1] of Holstein bulls.

Trait	Allele effect	P
Milk, kg	−18	0.16
Fat, kg	−0.4	0.42
Fat, %	+0.003	0.52
Protein, kg	−0.9	0.02
Protein, %	−0.003	0.10
Productive life, mo	−0.01	0.91
Somatic cell score	+0.001	0.95

[1]Daughter yield deviation adjusted for mean of parent evaluations of bull.

The linkage test was applied to the four specified heterozygous bulls, and the results are in Table 5. Only 2 of 28 tests were significant at $P < 0.05$. If the pleiotropic effect on protein yield indicated in Table 4 were real, a greater effect might have been expected for Table 5. The only significant effects in either table were for protein. However, for both pleiotropy and linkage, the association of the BLAD allele with protein performance was negative (as was the association with most other performance traits); therefore, elimination of the BLAD allele would seem to have no undesirable consequences. The relatively high frequency attained by the BLAD allele apparently resulted from its chance occurrence in a popular family, which included Carlin-M Ivanhoe Bell, a bull that was widely used because of his unrelated high merit for other traits.

Testing for the presence of the allele responsible for BLAD permitted the rapid control of BLAD in the US breeding population of Holsteins. The practice of the AI industry has been to consider carrier status as a variable when evaluating a bull that has already been sampled but not to sample carriers. Carrier bulls that are superior for other traits remain in AI service. Following the January 1995 evaluation, 44 carriers remained in active AI service. As shown in Table 6, those carriers were superior to other bulls (noncarriers and untested bulls) for all performance traits examined. Data such as these may have caused some breeders to conclude mistakenly that the BLAD allele has a positive relationship with performance traits. Of the 83 active AI bulls not reported as tested, none are likely to be carriers.

CONCLUSIONS

Because the test for determining the presence of the BLAD allele is accurate and relatively inexpensive, all bulls in active AI service or both parents should be tested for the allele. If females are not tested, pedigree examination through six or seven generations of males is probably required to reduce the chance of presence of the allele to 0%. For future undesirable recessives, the dairy industry would benefit by developing a procedure to encourage more complete reporting of animals tested before registration. Also, a procedure would be useful to identify animals that are considered to be free of an undesirable allele because both parents are free of the allele.

The ability to test for the BLAD allele has virtually eliminated it from active AI bulls of the future. No known carrier bulls have been sampled in recent years. Little evidence exists of an association of the BLAD allele with performance traits either through pleiotropy or linkage, and most associations were negative. Thus, the exclusion of bulls with the BLAD allele from progeny-test programs should not have

TABLE 5. Least squares solutions for effect of the allele for bovine leukocyte adhesion deficiency (BLAD) on granddaughter performance[1] of Holstein carrier bulls with ≥10 sons and numbers of bull sons tested for BLAD.

Trait	Allele effect for granddaughters of			
	Bell	Jesse	Secret	Bell Troy
Milk, kg	+20	−1	−43	+45
Fat, kg	+2.2	−2.1	−1.2	+1.1
Fat, %	+0.015	−0.021	+0.004	−0.007
Protein, kg	+1.5	−2.4*	−1.6	−0.1
Protein, %	+0.008	−0.024**	−0.002	−0.016
Productive life, mo	−0.31	−0.01	+0.45	+0.20
Somatic cell score	−0.03	−0.02	+0.01	−0.04
Sons tested for BLAD, no.	131	157	189	76

[1]Granddaughter yield deviation adjusted for mean of parent evaluations of son.

*$P < 0.05$.

**$P < 0.01$.

TABLE 6. Mean genetic merit of Holstein bulls reported during January 1994 as active AI according to carrier status for bovine leukocyte adhesion deficiency.

Trait	Carriers	Noncarriers or unknown
Bulls, no.	44	507
PTA for		
Milk, kg	546	517
Fat, kg	20.2	16.7
Protein, kg	17.5	15.8
Productive life, mo	1.20	1.14
Somatic cell score	3.20	3.20

any negative impact on genetic improvement for performance traits.

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