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Milk and fat yields decline in bovine leukemia virus-infected Holstein cattle with persistent lymphocytosis

(bovine major histocompatibility complex/economic loss)

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ABSTRACT **Effects of bovine leukemia virus (BLV) infection on milk and fat yields were studied by using data collected from Holstein cows over a 6-year period. Milk and fat yields in BLV-infected cows with persistent lymphocytosis (PL) declined significantly relative to their BLV-infected non-PL herdmates. Declines were most pronounced in cows older than 6 years. The estimated loss to the dairy industry due to PL is more than $42 million annually. A major histocompatibility complex class I (*BoLA-A*) allele that has been previously associated with resistance to PL was associated with longevity and realization of milk production potentials, indicating that genetic resistance to PL will have an economic benefit in herds where BLV is endemic.**

Bovine leukemia virus (BLV) is an exogenous retrovirus that is closely related to the human T-lymphotropic virus types I and II (1). BLV infection is endemic in the United States, with at least half of all dairy herds infected and often >70% of animals within these herds having antibodies to the virus (2, 3). A persistent B-cell lymphocytosis (PL) develops in 20–30% of infected cattle (4), and genetic resistance to PL maps closely to the class II genes of the bovine major histocompatibility complex, *BoLA* (5). Lymphosarcoma, the terminal stage of BLV infection involving the clonal transformation of infected B cells, occurs in about 1% of BLV-infected cattle and also appears to be under genetic control of the host (6).

The economic impact of BLV infection was estimated at $44 million in the United States in 1987 (7). Costs were largely associated with slaughterhouse condemnation of tumor-bearing carcasses and replacement of sick or dead animals. Subsequent studies (8, 9) suggest that the true cost of BLV infection may be greater due to losses in milk production. Cows with high genetic potentials for milk and fat yields have greater susceptibility to PL than cows with lower genetic potentials, but cows with PL do not produce yields of milk or fat according to their predicted genetic values (8). The present study was conducted to examine the effects of subclinical progression of BLV infection on milk production traits in individual cows, using data collected over a period of 6 years. An estimate of the economic impact of PL was made, and the relationship among animal age, PL, and *BoLA-A* allele frequencies was described in a herd with high prevalence of BLV infection.

MATERIALS AND METHODS

Experimental Animals and Data Collection. All cattle were reared and maintained at the University of Illinois dairy research facility. The data set used for the analysis of milk production traits included 626 lactation records on the first six lactations for 204 Holstein cows over a 6-year period. These 204 cows represented all animals in the herd with more than one lactation record during the 6 years. Records for milk and fat yields were 305-day twice-daily-milking mature equivalent (305-2×-ME). Each year in March (1985–1990), serological status to BLV and lymphocyte counts were determined for all animals in the herd. Seroreactivity to BLV was tested with an agar gel immunodiffusion (AGID) test (8). Leukocyte counts and differential blood cell counts were performed by standard hematological techniques (8), and animals were classified as hematologically normal or PL according to the European Community's Leukosis Key (10). All but 7 of the 204 cows were typed for *BoLA-A* (major histocompatibility complex class I) alleles. Parous and alloimmune typing reagents whose specificities were standardized to results of the Fourth International BoLA Workshop (11) were used for *BoLA-A* typing. Animal numbers decreased with age across all BLV infection categories due to culling, which was performed without knowledge of BLV infection status. An animal's genetic merit for a production trait was expressed as the estimated breeding value, where breeding value = 2 × transmitting ability. Estimates of transmitting ability were results of the July 1991 national genetic evaluation of dairy cattle conducted by the U.S. Department of Agriculture using an animal model that takes into account production records of all relatives (12).

Statistical Analysis. To estimate the effects of BLV infection on milk production over time, animals were grouped into three categories of BLV infection according to their infection status: seronegative, seropositive non-PL, or seropositive with PL (PL^+). Animals that were negative for antibodies to BLV antigens (gp51 and p24) for all AGID tests over the study period were classified as seronegative. A seropositive classification indicates at least one positive AGID test over the lifetime of this study. Animals classified as PL^+ converted from seropositive non-PL to PL during this study. In our analyses of the effects of BLV infection on milk production traits, individual lactation records were corrected for genetic merit by using breeding value as a covariable, because of the relationship between genetic potential and PL (8), to facilitate comparison of cows on an equal genetic basis. The effect of BLV infection on production traits was assessed by generalized least-squares analysis based on the following model:

$$y = xb + m + c + t + p + e,$$

where y = production trait, x = estimated breeding value for the production trait, b = regression coefficient of y on x, m = lactation effect (lactations 1–6), c = random permanent

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Abbreviations: AGID, agar gel immunodiffusion; BLV, bovine leukemia virus; PL, persistent lymphocytosis; PL^+, BLV-seropositive with PL; 305-2×-ME, 305-day twice-daily-milking mature equivalent.

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environment effect for repeated records within cow, t = treatment effect of growth hormone (cows treated or not treated; 38 lactation records were from 38 cows on a growth hormone trial), p = effect of number of years within BLV infection status (seronegative, seropositive non-PL, and PL$^+$ for a total of 14 levels as shown in Fig. 1*A*), and e = random residual with diagonal variance–covariance matrix structure. The variance ratio of residual variance to permanent environment variance used in the statistical test was 2.8125, based on the ratios of variance components for the national animal model evaluation in dairy cattle (12). Herd, year, and season, which are factors commonly fitted in models for dairy records, were not included in our model because the data were from one herd and because year and season did not have a significant effect for our data. Statistical tests were performed with PEST, a computing package for mixed models (13).

The relationship between the frequencies of *BoLA-A* alleles over time and BLV infection categories was analyzed by χ^2 tests (14–16). For each BLV infection category, the frequency of each *BoLA-A* allele was tested for differences between adjacent age groups in order to detect changes in gene frequencies as a function of the subclinical progression of BLV infection.

Estimation of Economic Effect. Estimation of economic loss was based on the total number of PL cows, price of milk, average milk yield, net income per cow, and loss in milk yield for PL cows as found in this research. The number of cows in the United States in 1991 was 9,990,000 (17), of which 93% were Holstein (18). At least 50% of Holstein herds are infected with BLV (3). Within infected herds, we assumed that 70% were infected and 20% of the infected animals develop PL (2, 3, 19). Therefore, a conservative estimate for the total number of Holstein cows with PL is (9,990,000)(0.93)(0.50)(0.70)(0.20) = 650,349. The national average milk yield per Holstein cow is 6810 kg (15,000 lb); the 1991 price for raw milk was \$0.27/kg (\$12.24/100 lb; ref. 17). Based on the above parameters, we estimate the gross income of milk production for Holstein cattle as (9,990,000)(0.93)(6810)(\$0.27) = \$17,082.81 million. The average operator's share labor and management income in Illinois was \$155 per cow in 1991 (20). This income is the net income reduced by a charge for unpaid family labor and interest on equity capital (20). We used this estimate for the national average of net income per cow.

RESULTS

Effects of BLV Infection on Milk Production Traits. Comparisons of milk production traits between seronegative and the other two BLV infection categories could be made only for the younger ages because nearly all animals that were not culled had seroconverted by 5 years of age. For each age category, milk and fat yields from seronegative animals were not significantly different from those of other herdmates; however, the number of observations in the seronegative group was relatively small. In contrast, after animals first tested positive for PL, milk and fat yields declined relative to their seropositive non-PL herdmates (Fig. 1). The relationship between PL and milk production traits was most pronounced after cows had PL for 2 years. For the first two lactations prior to the development of PL, these cows had about the same milk and fat yields as other cows of the same age (Fig. 1). After 1 year with PL, cows that remained in the herd experienced a precipitous and significant drop in milk yield and fat yield compared with their BLV-infected non-PL herdmates of the same age (Table 1). For non-PL cows older than 6 years, milk yield increased relative to the previous lactation (Fig. 1). This increase was most likely due to artificial selection—i.e., the retention of only the highest milk-producing cows in the herd. For example, the 27 non-PL

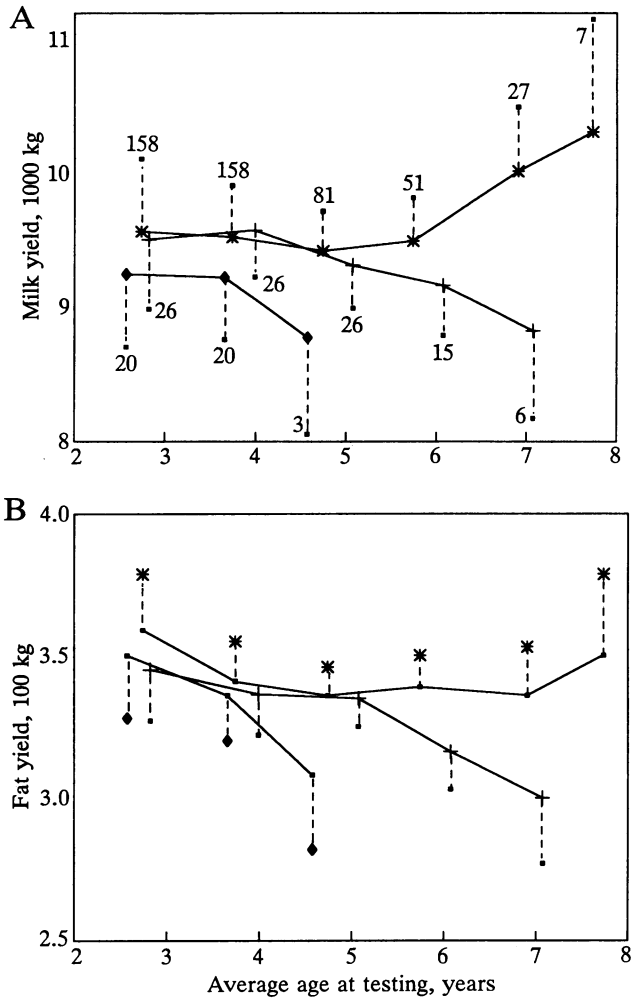

FIG. 1. Milk and fat yields (305-2×-ME) decline in cows that have had PL for ≥2 years. Milk (*A*) and fat (*B*) yields in seronegative (◆), seropositive non-PL (*), and PL$^+$ (+) cows according to animal age are shown. Symbol connected by dashed line is the corresponding standard deviation of the generalized least-squares mean. The number of cows for each point is indicated in *A*. The classification of BLV infection status is described in *Materials and Methods*. The first year of PL for all 26 PL$^+$ cows was 5 years of age on average. Lactation records in the two prior years when these animals were seropositive and non-PL are shown. Only one PL cow was seronegative 2 years prior to developing PL. Within the group classified as "seropositive non-PL," at 6 years of age, all 51 animals were seropositive. Prior to 6 years of age, the number of animals that were seronegative at the time of testing was 6 out of 81 (7.4%), 12 out of 158 (7.6%), and 35 out of 158 (22.2%), at 5, 4, and 3 years of age, respectively. Within each category of BLV infection and successive age group, animals are a subset of animals in the previous age group. The 305-2×-ME values for milk and for fat were corrected for breeding value, lactation number, and growth hormone treatment to evaluate the effects of BLV infection on milk production traits.

cows older than 6 years had an average milk yield (least-squares means) about 733 kg higher than the herd average. In comparison with non-PL cows, fat percentage in cows with PL was relatively unchanged due to comparable decreases in milk and fat yields (Table 1). The seronegative group had nonsignificantly lower milk and fat yields than their herdmates. The low average yield for the oldest seronegative cows (Fig. 1) resulted from three surviving cows with low yields and was not due to declining yields relative to the previous lactation. Among those three cows, milk yield actually increased for one cow, decreased for one, and was relatively unchanged for the other.

Table 1. Differences (Δ) between PL$^+$ and seropositive non-PL cows for milk and fat yields and fat percentage

Years with PL	ΔMilk, kg	ΔFat, kg	ΔFat percentage
2	−336	−23.5*	−0.10
3	−1204**	−38.4**	0.08

Differences in production traits (milk, fat, or fat percentage) were tested based on a generalized least-squares analysis using the model described in *Materials and Methods*. *, $P < 0.10$; **, $P < 0.05$.

Economic Loss Due to Decrease in Milk Yield for BLV-Infected Cows. The estimated annual loss in milk yield was 336 kg for cows that had PL for 2 years, and 1204 kg for cows that had PL for 3 years (Table 1). The estimated annual economic loss is ($0.27/kg)(336 kg) = $90.72 for cows with PL for 2 years, and is ($0.27/kg)(1204 kg) = $325.08 for cows with PL for 3 years (see *Materials and Methods*).

The annual economic loss to the dairy industry caused by PL was estimated by calculating the loss in milk yield for cows that had PL for 2 years or more, which is the time when milk yields start to decline (Fig. 1). Economic loss due to declined fat yield for PL cows was not estimated because the loss in fat yield is already taken into account by the loss in milk yield. We used the loss in milk yield for cows with PL for 3 years as an estimate of loss for cows with PL for >3 years. Such an estimate should be conservative because loss in milk for cows with PL becomes more pronounced over time (Fig. 1*A*). For U.S. Holstein cattle, 28.8% have four lactations (average 6 years of age) or more (21), and thus could have PL for ≥2 years. Among cows with four or more lactations, 42% had four lactations, and 58% had five lactations or more (21). The average loss for cows with PL for ≥2 years is estimated as (0.42)($90.72) + (0.58)($325.08) = $226.65. Then, the economic loss to the dairy industry due to PL is estimated as ($226.65)(0.288)(650,349) = $42,451,436, or about 0.25% of the gross income of milk production. Using the operator's share of labor and management income per cow in Illinois ($155) as a national estimate of the net profit per cow, the percent annual loss in net profit to the dairy industry due to PL is $42,451,436/[(9,990,000)(0.93)($155)] = 3%. Adding to the $44 million non-production loss estimated in 1987 (7), the total annual economic loss due to BLV infection in the United States is at least $86 million.

Relationship Between *BoLA-A* Alleles and BLV Infection Status. The phenotype frequencies of two *BoLA-A* alleles previously shown to be associated with resistance and susceptibility to PL were plotted against average age at testing in animals grouped by category of BLV infection and PL status (Fig. 2). The phenotype frequencies of all *BoLA-A* alleles observed in this herd were in accord with previous estimates (22). The frequency of the PL resistance-associated *BoLA-A14* allele (2) in any age group was higher in BLV-infected non-PL cattle than in BLV-infected PL cattle in the same age group ($P < 0.03$) and increased from 30% in 3-year-old cows to 52% and 59% in 7- and 8-year-old cows that were retained in the herd. In contrast, the frequency of *BoLA-A14* in cows with PL decreased from 7% in 3- to 5-year-old cows to 0% in cows that were ≥6 years old (Fig. 2). The frequency of the PL susceptibility-associated *BoLA-A12* allele (2) remained relatively unchanged in PL cows but decreased slightly in 6- and 7-year-old non-PL cows and was completely lost in 8-year-olds (Fig. 2). The relationship between *BoLA-A* allele frequencies and BLV infection over time, and between BLV infection and milk yield, imply an association of *BoLA-A* alleles with the full expression of milk and fat production potentials under conditions where BLV infection is prevalent. These results suggest that genetic resistance to PL is associated with longevity in this herd, where there is a high prevalence of BLV infection.

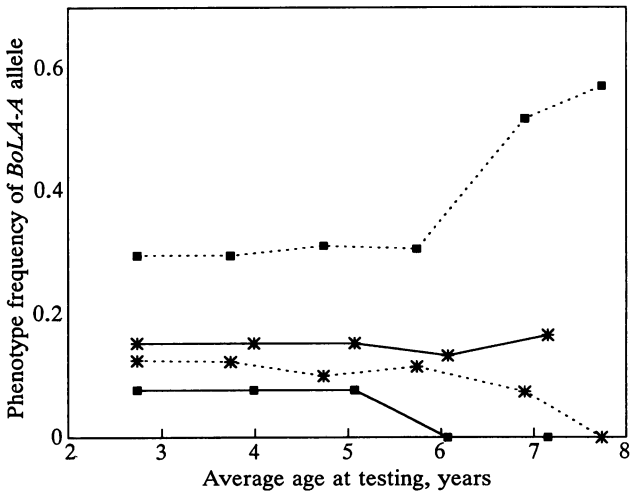

FIG. 2. The *BoLA-A14* allele increases in frequency over time in seropositive non-PL cows. The frequency of *BoLA-A14* in seropositive non-PL (· · ·■· · ·) and PL$^+$ (---■---) cows and the frequency of *BoLA-A12* in seropositive non-PL (· · ·*· · ·) and PL$^+$ (---*---) cows are shown. None of the other 15 *BoLA-A* alleles detected in this herd deviated appreciably in frequency with age. Numbers of animals in each BLV infection category are shown in Fig. 1*A*, except for the seropositive non-PL group, where seven animals did not have *BoLA-A* typing. This resulted in seven fewer observations in 3- and 4-year-old cows, and one fewer observation in 5-year-old cows when compared with numbers of animals in Fig. 1*A*.

DISCUSSION

Early attempts to quantify the economic impact of subclinical BLV infection focused on differences in milk production between seropositive and seronegative cattle (23, 24). The essential problem in interpretation or comparison of these studies is that "seropositive" animals are heterogeneous for disease status. For example, antibodies to BLV can be found in animals recently exposed to BLV but asymptomatic, in animals with PL (≥3 years old) and in animals with tumors (usually older than 6 years). Furthermore, the genetic potential for milk production was not taken into account in those early studies. As we have previously shown (8), genetic merit is correlated with susceptibility to BLV infection and PL. Therefore, inconsistent results between these early studies are not surprising. Our approach for analyzing the effect of BLV infection on milk production was to subdivide the seropositive animals into PL$^+$ and non-PL categories and to take into account the genetic potential for various measures of milk production for each animal. This approach resulted in a clear picture of the effects of subclinical BLV infection on milk production. It is noteworthy that the 1991 milk price used for the estimation of economic loss was the lowest in the period 1989–1991. If the 1990 milk price ($0.303/kg) is used, the loss in milk due to PL is at least $47 million, and the total loss due to BLV infection is raised to at least $91 million per year. Although the mechanism by which the PL state reduces milk and fat yields is unknown, altered energy balance due to the maintenance of lymphocytosis or BLV infection of mammary epithelial cells might provide an explanation for these findings.

Our earlier study (8) indicated that PL did not have an adverse effect on fat yield but that fat percentage was reduced due to the increase in milk yield. In contrast, the present study showed that milk and fat yields were reduced after the first year of PL. An explanation of differences might be that few PL cows in the previous cross-sectional study had PL for ≥1 year.

A complex relationship exists among genetic merit, milk production, *BoLA* genotype, and susceptibility to PL. Al-

though the association between PL susceptibility and *BoLA* genotype is stronger for class II genes (5), the relationship to the *BoLA-A* class I alleles was presented because most data were collected prior to the development of methods to discriminate *BoLA* class II alleles. Because there is strong linkage disequilibrium between *BoLA* class I and class II alleles within Holstein cattle (ref. 25; H.A.L., unpublished data), the comparison of *BoLA-A* frequencies is still informative when one considers that the initial discovery of the association between *BoLA* and PL was made by using serological *BoLA-A* typing (2). It is known that *BoLA*-mediated resistance to PL behaves as a dominant Mendelian trait (5). Further, it is known that genes affecting milk production are closely linked to the *BoLA* system (26, 27). From these data, it is impossible to distinguish whether susceptibility to PL is a consequence of the physiological stress imparted by high milk production or whether there are separate genes that influence milk production and susceptibility to PL that are closely linked on the same chromosome. Because there are many high-producing cows that do not develop PL, we favor the interpretation that different genes on bovine chromosome 23 control resistance to PL and milk production. For example, the gene encoding prolactin, an important protein involved in milk secretion, is 4 centimorgans from *BoLA-DRB3* (27). Sorting out the various genetic, physiological, and environmental factors that influence milk production in BLV-infected cattle will serve as an important model for studying the role of other subclinical infections on animal production efficiency. Moreover, given the economic consequences of PL, strategies aimed at reducing the incidence of BLV infection, for which there is no commercially available vaccine, will yield significant monetary returns to dairy producers.

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