Factors Associated with *in utero* or Periparturient Transmission of Bovine Leukemia Virus in Calves on a California Dairy

Marie-Liesse G. Lassauzet, Mark C. Thurmond, Wesley O. Johnson and Charles A. Holmberg

ABSTRACT

A three-year prospective study involving 143 calves born from infected cows was undertaken on a California dairy to evaluate possible factors of the dam associated with bovine leukemia virus infection *in utero* or during the periparturient period. *In utero* or periparturient infection occurred at a rate of 4.8% and was more likely in calves born to cows with an average peripheral blood lymphocyte count during pregnancy greater than 12,000 cells/μL ($p = 0.043$) or in calves born to cows that developed malignant lymphoma ($p = 0.00004$), but not in calves born to cows with p-24 antibodies ($p = 0.675$).

RÉSUMÉ

Une étude prospective d'une durée de trois ans portant sur 143 veaux nés de vaches atteintes de leucose bovine, a été entreprise dans un troupeau californien. Le but de l'étude était d'évaluer les facteurs maternels possiblement associés à l'infection par le virus de la leucose bovine, *in utéro* ou pendant la période péripartum. Le taux d'infection *in utéro* ou péripartum s'établissait à 4.8 %. L'infection survenait surtout chez les veaux nés de vaches ayant un comptage lymphocytaire moyen plus élevé que 12 000 cellules μL ($p = 0.043$) dans le sang périphérique au cours de la gestation, ou chez les veaux nés de vaches ayant développé un lymphome malin ($p = 0.00004$), mais non chez les veaux nés de vaches ayant des anticorps (p-24 ($p = 0.675$). (*Traduit par Dr Nicole Hamelin*)

INTRODUCTION

Bovine leukemia virus (BLV), a type-C retrovirus, is the causative agent of enzootic bovine leukosis (1). Early transmission of the virus from the dam to its progeny can occur *in utero*, during, or just after parturition (2). In commercial herds, *in utero* infection occurs in 3 to 6% of calves born from infected cows (3-8). Although male calves may be at a higher risk of *in utero* infection than female calves (4), no characteristic of the infected dam has been found to be associated with *in utero* infection (4,9). Knowledge of such characteristics, if they exist, might permit control of *in utero* infection by removing cows at a high risk of having a fetus infected *in utero*. Knowledge of which dams are more likely to give birth to calves infected *in utero* also may be used to reduce postnatal transmission. Calves with a high probability of being infected at birth could be segregated or removed from the herd, thereby reducing risk of infection to other calves. Currently, transfer of embryos from infected donors to noninfected recipients is the only method available to prevent *in utero* transmission (10,11).

Control programs need to be efficient and easy to implement. Taking precolostral samples on all calves born from infected cows to identify those infected *in utero* is logistically very difficult and may interfere with management of commercial herds. A less constraining method to identify calves infected *in utero* would, therefore, be highly desirable.

The objectives of this study were to characterize the rate of *in utero* or periparturient (IUP) infection with BLV in dairy calves on this farm, to examine certain dam characteristics possibly associated with that rate and to evaluate whether postnatal repeated bleedings could be used to identify *in utero* infected calves under field conditions.

MATERIALS AND METHODS

POPULATION STUDIED

The calves studied were born between January 1984 and June 1987 on a 210-cow dairy located in the Central San Joaquin Valley of California and managed as a typical feedlot dairy (12,13). Prevalence of BLV infection in the whole herd averaged 37.8% over the three-year period of the study (13). Within 12 h of birth, calves were fed 2 L of colostrum from their own dams, or given Genecol 99® and fed 1 to 2 L of pooled colostrum. Female calves were retained in the herd to provide replacements and most bull calves were sold. Within 12 h of birth, calves were put in individual hutches. Once in hutches, calves were fed 2 L of pooled colostrum twice a day for two days.

BLOOD COLLECTION AND EXAMINATION

Whole blood was collected from newborn calves usually within 1 wk of birth during bi-weekly visits to the herd; if calves were born during these regular visits, precolostral samples were taken at that time. Blood was then collected from all calves every 2 to 3 wk until 90 days of age, after which time blood was collected every three months. Cows were bled quarterly

Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California, 18830 Road 112, Tulare, California 93274 (Laussauzet, Thurmond, Holmberg) and Division of Statistics, University of California, Davis, California 95616 (Johnson). Present address of Dr. Lassauzet: Ciba-Geigy, Biovet Unit, Centre de Recherches Agricoles, CH-1566 Saint-Aubin FR, Switzerland.

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and when examined by the herd veterinarian during weekly visits for reproduction monitoring. Serum was separated by centrifugation and stored at −80°C. Blood was also collected from adult cattle at each quarterly bleeding into tubes containing sodium ethylenediaminetetraacetate.

Agar-gel immunodiffusion (AGID) was used to detect gp-51 and p-24 antibodies using the gp-51 antigen (Leukassay-B kit, Pitman-Moore Inc., Washington Crossing, New Jersey) and a dual gp-51/p-24 antigen (Dr. Janice Miller, US Department of Agriculture, Ames, Iowa, and Dr. Charles Holmberg, UC Davis, Tulare, California). The test procedure for the detection of gp-51 antibodies was as previously described (14). Detection of p-24 antibodies was made using petri dishes 100 mm in diameter containing 16 mL of agar. The concentration of antibodies present in the serum was approximated by categorizing from 1 (lowest concentration) to 4 (highest concentration) according to the shape of the precipitating line and its distance from the perimeter of the antigen well (National Veterinary Services Laboratory, Ames, Iowa).

Because the AGID test is not very sensitive for the detection of p-24 antibodies (15), animals were considered p-24 positive even if p-24 antibodies were not detected at all bleedings. Cows that had p-24 antibodies detected at least twice were classified as positive.

The number of leukocytes per μL of blood was counted using a Coulter counter (Model ZM, Coulter Electronics Limited, Luton, England). Differential counts were done by counting the first 100 leukocytes on peripheral blood smears prepared with a modified Wright's stain and then multiplying the proportion of cell type by the total number of leukocytes. For each calf, the average peripheral blood lymphocyte count per μL of blood (PBLC) of its dam during pregnancy was obtained by summing values of PBLC taken during the pregnancy and dividing by the number of values.

DETERMINATION OF INFECTION STATUS

A calf was classified as IUP infected if precolostral serum was positive for gp-51 antibodies, or, in the event that no precolostral serum was available, if gp-51 antibody concentrations of all postcolostral sera were categorized as 4 for all tests during the first six months of life. The basis for this classification was that, in a previous study, calves that maintained consistently high titers to BLV had antibodies detected precolostrally (16).

FACTORS STUDIED AND STATISTICAL METHODOLOGY USED

Factors hypothesized to be associated with IUP infection were the average PBLC of the dam during pregnancy, presence of detectable p-24 antibodies in serum of the dam on at least two tests, and presence of malignant lymphoma (ML) in the dam within 18 months postparturition. Fisher's exact test and logistic regression were used to determine whether any of the three factors were associated with IUP infection (17). Computations were done using BMD4F and BMDPLR (18). Chebychev's inequality was used to obtain a confidence interval for the proportion of cases of IUP infection attributable to the presence of ML in dam (17).

RESULTS

Between January 1, 1984 and June 30, 1987, 143 calves were born from infected cows. Two died before their IUP infection status could be determined. Of the 141 calves available for analysis, six had antibodies constantly detected at a level of 4 for the first six months of life. One of the six was sampled precolostrally and had antibodies detected (calf born in January 1985, Table I). The corresponding rate of IUP infection was 4.3% (6/141).

Information on the dams of the six IUP calves is reported in Table I. Three of these dams had an average PBLC during pregnancy greater than 12,000 cells/μL (Table I). Of those three cows, one was condemned at slaughter for ML with no histopathology confirmation, one died of ML with histopathological confirmation and one died of a ruptured spleen and was diagnosed as having lymphoid leukemia (Table I). Prior to death, the cow with a ruptured spleen had a total leukocyte count greater than 200,000 cells/μL, of which 84% were small lymphocytes. Gross examination of the carcass revealed enlarged hemal lymph nodes throughout the body. This cow was diagnosed as having lymphoid leukemia, but absence of recognizable lymphomatous lesions made the diagnosis of ML uncertain. During the study period, an additional cow (not

TABLE I. Information on dams of calves that were classified as infected *in utero* or periparturition

Calving date (month/year)	Removal date (month/year)	Cause of removal (age at removal or at end of study)	p-24 status	Duration of infection	Average PBLC[a] during pregnancy
03/85	01/86	Lymphoid leukemia (5 years)	positive	> 2 years	13,420
08/85	02/87	Mastitis; condemned for malignant lymphoma (3.5 years)	positive	> 3 years	33,258
01/85	Still in herd	Had another noninfected calf (6.5 years)	negative	> 3.5 years	6,159
03/87	04/87	Mastitis (5.5 years)	positive	> 3 years	4,229
02/87	Still in herd	Purchased in February 1987 (3 years)	positive	> 4 months	3,944
07/86	03/87	Malignant lymphoma (4.5 years)	positive	> 3 years	20,018

[a]PBLC: peripheral blood lymphocyte count per μL of blood

TABLE II. Relationship between *in utero* or periparturient (IUP) infection with bovine leukemia virus and average peripheral blood lymphocyte count per μL (PBLC) of dam during pregnancy

Average PBLC of dam during pregnancy	IUP infection: Yes	IUP infection: No	Total
	6	135	141
< 12,000	3	117	120
≥ 12,000	3	18	21
Fisher's exact test p = 0.043			
< 10,000	3	111	114
≥ 10,000	3	24	27
Fisher's exact test p = 0.084			
< 8,000	3	102	105
≥ 8,000	3	33	36
Fisher's exact test p = 0.174			

presented in Table I) was believed to have ML. At her last calving, she had a bull calf that was sold soon after birth. She was condemned at slaughter for ML with no histopathological confirmation.

When dam PBLC was examined for an association with IUP infection, dam PBLC was categorized into low, medium and high groups. Three cut-off points were considered, namely 8,000, 10,000 and 12,000 per μL. Estimated probabilities of IUP infection for calves born to cows with low PBLC ranged from 0.025 to 0.029 (3/120 to 3/105) and for high PBLC, from 0.08 to 0.14 (3/36 to 3/21) (Table II). Statistical significance of an association between IUP infection and dam PBLC ranged from p = 0.043 for a cut-off point of 12,000 per μL, to p = 0.174 for a cutoff point of 8,000 per μL, using Fisher' exact test (Table II). The relative risk of IUP infection for calves from cows with PBLC ≥ 12,000 cells/μL compared to those from cows with PBLC < 12,000 cells/μL was 5.7 [(3/21)/(3/120)]. A logistic regression model, with PBLC left uncategorized, also showed a statistically significant association between IUP infection and PBLC (p = 0.048). The probability of IUP infection for a calf born to a dam with a given PBLC during pregnancy could be predicted before its birth using the following model:

$$\text{Probability of IUP infection} = \frac{\exp(4.08 - 0.0001*\text{PBLC})}{1 + \exp(4.08 - 0.0001*\text{PBLC})}$$

The presence of p-24 antibodies in the dam, however, was not found associated with IUP infection. The observed proportion of IUP infected calves was 0.05 (5/101) for those born to cows with p-24 antibodies and 0.025 (1/40) for those born to cows without p-24 antibodies (p = 0.675).

The IUP infection was found to be associated with the presence of ML in the dam within 18 months postparturition (p = 0.00004). The observed proportion of IUP infected calves born to cows suspected of having developed ML was 1.00 (3/3), while that for IUP infected calves born to cows that did not develop ML was 0.02 (3/138). The observed proportion of IUP infection attributable to presence of ML in dam was 0.98 (1.00–0.02) or 98%, and a conservative 95% confidence interval based on a Chebychev inequality was (0.82–1.00) (17). The estimated relative risk was 46 [(3/3)/(3/138)].

Forward stepwise logistic regression was used to obtain a model for the probability of IUP infection as a function of all three hypothesized factors, presence of p-24 antibodies in the dam, average dam PBLC during pregnancy, and presence of ML in the dam. The chi-square p-values to enter the model for presence of p-24 antibodies, average PBLC, and presence of ML were 0.494, 0.048 and < 0.0001, respectively. Once ML status entered the model, the p-value of the chi-square-to-enter for average PBLC was 0.217; thus, the final model included only ML status.

DISCUSSION

The IUP infection rate estimated in our study (4.3%) is within the range of the *in utero* infection rates observed in previous studies of 1.2%–6.4% (4–9), and supports the belief that, under natural conditions, *in utero* infection is infrequent.

In a previous report (16), the method of examining serological results from repeated bleedings was proposed as a means of identifying calves that were infected *in utero*; all calves with persistent antibody titers had antibodies detected precolostrally. Because our approach to identify IUP calves was based only on results of that report, the question was raised whether this approach could overestimate the true IUP infection rate if non-IUP calves can exhibit high persistent titers. If this were true, calves, while possessing a high colostral BLV antibody titer, would have to become infected some days or months after birth and then rapidly produce a high level of infection-induced antibodies. Such a hypothesis contradicts previous results. Colostral antibodies to BLV have been found to have a protective effect against infection (14,19), and one calf inoculated after birth while having colostral antibodies first exhibited declining titers before producing infection-induced antibodies (19). Moreover, in another study, of 14 calves born from an infected dam, infected naturally, and showing initially declining colostral titers then increasing infection titers, five were tested precolostrally and found to be negative (20).

In the same report (16), some calves with a positive precolostral sample had postcolostral titers that decreased and then either increased or remained negative. One possible explanation is that "precolostral" samples for those calves were obtained after colostral intake. Others have reported that, in field studies, such an error can occur (4,5). If calves that had a positive precolostral sample can truly exhibit decreasing postcolostral titers, then our approach may have underestimated the true IUP infection rate since calves with declining titers were not classified as IUP infected.

The IUP infection was not found to be associated with the presence of p-24 antibodies in dams but was found to be associated with the average PBLC of the dam during pregnancy (Table II). The proportion of IUP infected calves born to dams with PBLC ≥ 12,000 per μL was much lower (3/21 or 14%) than that found in a similar study (4/5 or 80%) using a measure of persistent lymphocytosis (21). Explanations for

such a wide difference might be different methods of classifying *in utero* infected calves and/or persistent lymphocytosis cows.

In utero infection rates of 18% and 20% have been observed in herds with a high incidence of lymphosarcoma (22,23). The relatively high proportion of *in utero* infection in fetuses from cows with ML, 2/5 (24) and 5/15 (25), could be explained by the association found here between IUP infection and development of ML in the dam. Because cows with ML usually have high PBLC (22), it is possible that such cows would be more likely to infect their progeny because of an elevated PBLC and/or because of the presence of tumorous or pretumorous lesions in the endometrium or the placenta (25,26). The weaker associations found between IUP infection and average PBLC when cut-off points were selected at 8,000 and 10,000 per μL might be explained by the fact that the three cows with ML had an average PBLC $\geq$ 12,000 per μL. Because of the small number of IUP infections, it was not possible to examine the interaction between the presence of ML in the dams, average PBLC and the presence of p-24 antibodies.

Although the association with IUP infection was weaker for dam PBLC than for dam ML status, the use of dam PBLC monitoring may have considerable merit in programs to control and reduce BLV infection because knowledge of the average PBLC of the dam is readily available, while knowledge of presence of ML is not. Cows identified as having PBLC $\geq$ 12,000 per μL, for example, could be managed to calve in an isolated area, thus minimizing possible transmission to other neonates in maternity pens. Calves born to cows with high PBLC could be removed from the herd, or segregated from other calves for about three months, time at which it could be determined whether antibodies were colostral in origin or due to infection (20). Systematic removal of calves born to cows with PBLC $\geq$ 12,000 per μL would not be justified, however, because the attributable risk of infection was only 12% (3/21-3/120).

One cow was believed to have died of lymphoid leukemia, with enlarged hemolymph nodes throughout the body. She was classified as a case of ML (Table I) because hemolymph nodes might be early localizations of tumors (27). However, if this cow were deleted from analysis, a significant association between IUP infection and the development of ML in the dam was still found ($p = 0.001$).

In spite of possible misclassification of dam tumor status, the association observed between IUP infection and development of ML in the dam was very strong (relative risk = 46), and based on a large number of animals. It is hoped that results of our study will stimulate more elaborate further research to identify mechanisms of viral-induced tumor development by an intensive follow-up of dams that gave birth to an infected calf.

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