Protection of Colostral Antibodies Against Bovine Leukemia Virus Infection in Calves on a California Dairy

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ABSTRACT

A three-year prospective study involving 244 calves was undertaken on a California dairy to evaluate the protective role of colostral antibodies against bovine leukemia virus (BLV) infection in calves. Calves were followed from birth to the time they left their individual hutch (TLIH), at about 90 days of age. The probability of being infected at TLIH and the daily risk of infection between birth and TLIH were modelled using the logistic and the Cox models, respectively. Calves with no detectable antibodies during the first week of life were up to 2.00 and 2.75 times more likely to be infected at TLIH compared to calves with low and high concentrations of antibodies during the first week of life, respectively ($p = 0.01$). When the daily risk was modelled, calves without antibodies at the estimated day of infection were up to 3.4 and 11.6 times more likely to become infected than calves with low and high concentrations of antibodies on that day, respectively ($p < 0.001$). Results indicated that calfhood infection may be reduced by about 45% through the feeding of colostrum with BLV antibodies. Further reduction in infection may be possible by feeding calves milk powder, milk replacer, and/or milk from noninfected cows. Results also indicated that quantification of the effect of a time-dependent risk factor, such as colostral antibody concentration, might be affected if treated as a fixed factor.

RÉSUMÉ

Cette étude s'étalait sur une période de trois ans, impliquait 244 veaux d'un troupeau laitier de la Californie et consistait à évaluer le rôle protecteur des anticorps colostraux contre l'infection des veaux par le virus de la leucémie bovine. Les auteurs effectuèrent à cette fin le monitorage des veaux, de leur naissance jusqu'au moment où ils les enlevèrent de leurs cages individuelles, vers l'âge d'environ 90 jours. À l'aide des modèles logistique et Cox, ils profilèrent aussi la probabilité d'une infection, vers l'âge de 90 jours, et son risque quotidien, entre la naissance et l'âge précité. Les veaux dépourvus d'anticorps décelables, durant la semaine ultérieure à leur naissance, se révélèrent de 2,00 à 2,75 fois plus susceptibles d'être infectés, vers l'âge de 90 jours, comparativement à ceux qui en possédaient peu ou beaucoup ($p = 0,01$). Le profil du risque quotidien d'infection révéla que les veaux dépourvus d'anticorps, au jour approximatif de leur infection, s'avéraient de 3,4 à 11,6 fois plus susceptibles de devenir infectés que ceux dont le titre d'anticorps était faible ou élevé, ce jour-là ($p < 0,001$). Les résultats de cette expérience révélèrent la possibilité de réduire d'environ 45% l'infection des veaux, pourvu qu'on leur donne du colostrum porteur d'anticorps contre le virus précité. Cette réduction peut même s'accentuer, si on donne aux veaux du lait en poudre, un substitut du lait et/ou du lait de vaches saines. Les résultats révélèrent aussi qu'on peut affecter la quantification de l'effet d'un facteur de risque temporel, comme la teneur du colostrum en anticorps, si on le considère comme un facteur stable.

INTRODUCTION

Postnatal transmission of bovine leukemia virus (BLV) in calves may occur around parturition, via colostrum or milk, by contact with infected cattle, or by iatrogenic means (1). The importance of colostrum and milk as a means of transmission is not well known. Infection from milk or colostrum has been perceived as minimal by some (2,3) and high by others who consider it to be a significant form of transmission (4,5). Differences in results of these studies may reflect different herd characteristics and/or management factors, such as density of infected calves, prevalence of infection in the milking cows, housing of calves, colostrum management and other factors which could affect the rate of BLV infection in calves. As a result, it is still not known whether, in the control of BLV

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calfhood infection, one should recommend to feed calves colostrum with BLV antibodies in an effort to control subsequent infection or whether such a recommendation would increase the rate of infection.

In California dairies, calves are usually raised in individual hutches for the first three months of life. Such a type of housing permits the study of calfhood infection by means other than through contact with infected calves. The objectives of this study were to estimate the rate of BLV infection in dairy calves while housed in individual hutches and to determine whether colostrum from BLV-infected cows protected calves from infection or whether its consumption increased the risk of infection.

MATERIALS AND METHODS

POPULATION STUDIED

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 California feedlot dairy (6). Within 12 h of birth, calves were either fed 2 L of colostrum from their on dam, or given commercial immunoglobulins per os (Genecol 99^{R}) 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 shortly after birth. Within 12 h of birth, calves were put into individual hutches placed about 1 m apart where they were fed 2 L of pooled colostrum twice a day for two days, after which time they were fed twice daily with 2 L of a combination of bulk tank milk and powdered milk at a ratio of 1:1. Alfalfa hay and a grain mix were provided after four days of age. At about 90 days of age, calves were placed in a pen with seven to ten other calves. Calves were weaned at about six months of age.

BLOOD COLLECTION AND EXAMINATION

Whole blood was collected from calves usually within 1 wk of age. Precolostral samples were taken from calves born during bi-weekly visits to the herd. Blood was collected from calves every 2 to 3 wk until they left the hutch, after which time blood was collected every three months. Serum was separated by centrifugation and stored at -80°C.

Agar gel immunodiffusion (AGID) was used to detect antibodies to the gp-51 antigen of BLV (Leukassay-B kit, Pitman-Moore Inc., Washington Crossing, New Jersey). The test procedure was slightly different from the protocol described by Miller and Van der Maaten, and Nakajima *et al* (7,8). The agar gel was made with 1% agarose and 8.5% NaCl using distilled water. Glass plates measuring 100 by 80 mm and containing 21 mL of agar were used. Wells were 4 mm in diameter and at a distance of 5 mm from each other. Antigen was placed in the central well, the reference positive serum in two opposing wells, and sera to be tested in the four remaining wells. Plates were incubated 48 h at 25°C in a humified chamber before being viewed. A line of identity between antigen and antibody was interpreted as a positive result. The concentration of antibodies present in the serum was classified 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).

DETERMINATION OF AGE AT INFECTION

Calves with a precolostral serum positive for gp-51 antibodies or with all postcolostral sera categorized as 4 during at least the first six months of life were classified as infected *in utero* or shortly after parturition (9,10). Those calves were not considered in this study.

For calves that became infected after parturition, the exact age at infection, expressed in days, could not be determined. This is because calves were not bled daily and because the serological test fails to detect infection until antibody concentration reaches a detectable level. Using results from a previous study that estimated time-to-seroconversion following experimental BLV infection (11), it was assumed that infection, when viewed retrospectively, could have occurred between 110 days prior to the last negative test (tl) and seven days prior to the first positive test (t2). All possible ages at infection within that time interval (tl-110, t2-7) were assumed, *a priori*, to be equally likely. In other words, the prior probability function of the age at infection was assumed constant over that range of age values. Using the knowledge of time-to-seroconversion following experimental infection, the posterior probability function for the age at infection for an animal that seroconverted between tl and t2 could be obtained as described previously (11). Such functions allowed us to derive the "most likely" age at infection for a calf that seroconverted (11). In addition, the probability of becoming infected before a specific time of interest could also be derived (11). In the present study, the specific time of interest was the age at which a calf was moved from its hutch. The probability of becoming infected was computed for the period from birth to the age at which calves were removed from the hutch.

The ages at infection for calves that did not receive colostral BLV antibodies and that seroconverted were estimated using the above method. The same method was used also to determine the most probable age at infection for calves that received colostral antibodies before becoming infected and that were observed seronegative for at least two months after the last positive test of colostral BLV antibodies. Those calves were not considered as having been infected while having colostral antibodies (12). The limit of two months was selected because, in one study of a calf infected after birth, the time between the last colostrum-related positive test and the first infection-related positive test was two months using the AGID test (12).

For calves that received BLV colostral antibodies and were not observed seronegative for at least two months after the last test with the lowest colostral antibody concentration (CAC), age at infection was more difficult to estimate because passive antibodies could not be differentiated from infection-induced antibodies. Thus, it was assumed that those calves could have been infected while having colostral antibodies. Special attention was given to calves that were not observed negative two months follow-

ing the last test with lowest CAC. These types of calves were referred to as SU (status uncertain) calves. Two alternative methods were used to determine age at infection for SU calves that did not show a different rate of decay of colostral BLV antibodies compared to noninfected calves. One consisted of choosing t1 to be seven days after birth and the other consisted of choosing t1 to be the age at the last negative test or, if not available, the age at the last test with lowest CAC. For both methods, t2 was the age at the first test showing an increase in antibody concentration. The first method tends to overestimate the probability of infection at younger ages and, therefore, to overestimate the probability of infection during times when calves still have colostral antibodies. The second method tends to overestimate the probability of infection at older ages.

For SU calves that showed a different rate of decay of colostral BLV antibodies, t1 was chosen to be seven days after birth and t2 was the age at the first test where the CAC was significantly greater than the one expected for noninfected calves (10).

DESCRIPTIVE STATISTICS

Monthly cumulative hazards of infection and monthly average of daily prevalence rates were computed to provide an overall view of prevalence and risk of BLV infection for calves housed in hutches. To obtain monthly cumulative hazards, daily hazards were first obtained by summing conditional probabilities of becoming infected on a given day and dividing by the number of animals estimated to be at risk of becoming infected at the beginning of that day. Monthly cumulative hazards were derived by summing daily hazards over a given month.

To obtain monthly averages of daily prevalence rates, daily prevalence rates were computed by summing the estimated number of infected animals in a day and dividing by the total number of animals present on that day. Monthly averages of daily prevalence rates were computed by summing daily prevalences over a given month and dividing by the number of days in that month.

FACTORS STUDIED AND STATISTICAL METHODOLOGY USED

Two approaches were used to analyze the data. One consisted of estimating the probability of a calf being infected by the time it was moved from its hutch. Factors hypothesized to be associated with that probability were dam infection status and value of CAC during the first week of life (CAC1). The other approach used was more dynamic and consisted of modelling the age at infection, using survival techniques. Factors hypothesized to be associated with age at infection were dam infection status and CAC at age of infection.

For the first approach, the percentages of calves that became infected in hutches were computed for each dam infection status and each CAC1. The probability that a calf became infected while housed in a hutch was obtained by summing daily ordinates of the infection probability curve for all days spent in the hutch. Logistic regression was used to estimate whether the probability of infection before removal from a hutch was associated with dam infection status and/or CAC1. In this case, the responses were not zeros and ones, as would normally be the case for a logistic regression analysis, since it was not known exactly when a calf became infected. This analysis, therefore, used the estimated probability of infection as the response. The likelihood function was then obtained in the same way as that for a standard logistic regression. Computations were done using BMDP3R (13).

For the second approach, the most likely age at infection for a given calf was obtained from the estimated probability curve for age at infection. The Cox model was used to test the hypotheses that dam infection status and the time-dependent variable CAC were associated with the most likely age at infection (14). Computations were done using BMDP2L (13). The age at which CAC changed from a concentration level j to level j-1 was chosen to be the midpoint of the interval between the age at the last test at level j and the age at the first test at level j-1. A few calves were not tested while their CAC was at level j and only a drop from a level of j+1 to a level of j-1 was observed. For these calves, the age at level j was selected to be the median estimated duration of stay at level j for noninfected calves (10). For some analyses, CAC was categorized as low (coded 1) for a concentration of 1 or 2 and high (coded 2) for a concentration of 3 or 4.

RESULTS

Information on dam infection status and CAC1 (negative, low, high) was available for 308 of 359 calves born between January 1, 1984 and June 30, 1987 (Table I). Information was lacking on dam infection status for four, on CAC1 for 42, and on both dam infection status and CAC1 for five calves. About 16% (20/129) of calves born to infected cows did not have detectable colostral antibodies to BLV in their serum, while about 29% ([36 + 16]/179) of calves born to BLV-negative cows and presumably receiving pooled colostrum, had colostral antibodies to BLV.

Monthly cumulative hazards of infection ranged between 0 and 0.287 and average monthly prevalence in hutches ranged between 0 and 0.342 (Fig. 1). Some time-clusters of calfhood infection were observed with the greatest peak of new infections observed around December 1986 (0.287) (Fig. 1).

Sixteen calves were classified as SU calves which, for most, was because they were bled at an interval greater than two months and, therefore, could not qualify under the criterion of being seronegative for at least two months (Table II). Information on CAC1, dam average peripheral blood lymphocyte count per μL of blood

TABLE I. Status of Infection with Bovine Leukemia Virus (BLV) in 308 Cows and Concentration of Colostrom-derived BLV Serum Antibodies During the First Week of Life in their Calves Born Between 1 January 1984 and 30 June 1987 on a California Dairy

Colostral Antibody Concentration	Dams		
	Not Infected	Infected	Total
Negative	127	20	147
Low	36	45	81
High	16	64	80
Total	179	129	308

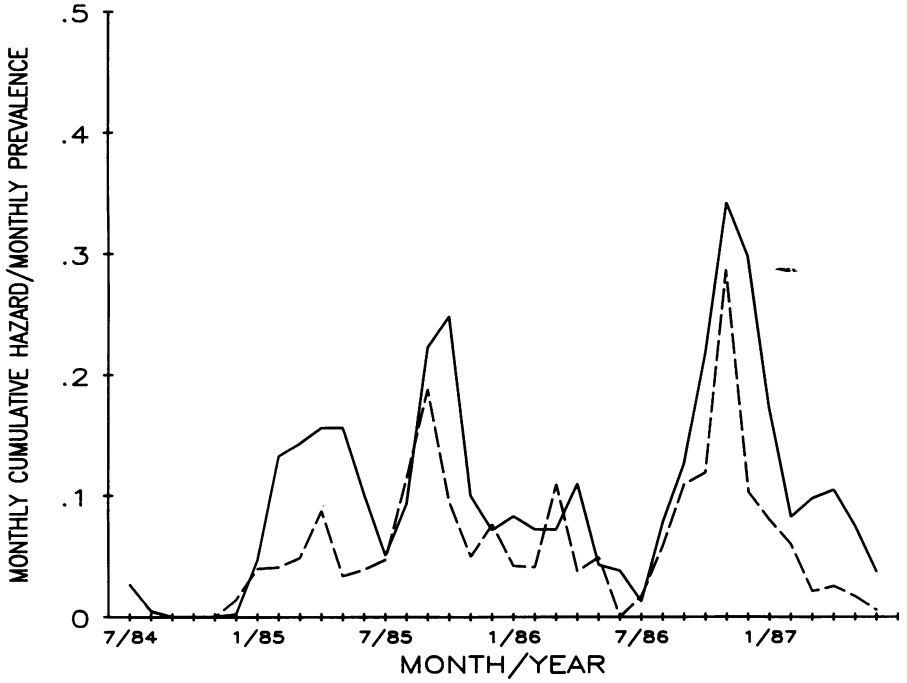

Fig. 1. Monthly cumulative hazards (— —) and average monthly prevalence of BLV (—) in calves housed in hutches.

(PBLC) during pregnancy, dam infection status, age at the last test with lowest CAC and age at the first test with an increase in titer, is provided in Table II for the 16 SU calves. Except for calf 428, rate of decay was similar for SU calves and noninfected calves. For that calf, age at infection was estimated to be between birth and 135 days, the time at which its CAC level was still equal to 3 (Table II) and, thus, above the CAC levels of noninfected calves.

For the remaining 15 SU calves, probability curves of age at infection were estimated using the two methods described previously. As previously mentioned, estimates of the most likely age at infection were less using the first method than using the second method (Table II). For example, the most likely age at infection for calf 203 was 44 days by the first method and 100 days by the second method (Table II).

Among the 359 calves born during the study, 66 either died before they left their hutch, or had not yet been moved to a group pen by the end of the study. Among the remaining 293, 17.4% (50.85/293) were estimated to have been infected by the time they were moved to group pens; this included calves classified as infected *in utero* or shortly after parturition. Information on dam infection status and CAC1 (negative, low, high) was available for 244 calves, not including the six calves classified as infected *in utero* or early *postpartum*. All further analyses were done on these 244 calves.

When the first method to estimate age at infection was used for the 15 SU calves, 22.4% ((3.72 + 23.63)/(16 + 106)) of calves that had a negative CAC1 became infected while housed in hutches, compared with 12.4% ((2.76 + 3.81 + 6.95 + 1.6)/(30 + 24 + 52 + 16)) of calves that had a positive CAC1 (Table III). Logistic regression was then used to analyze the data. Results of analysis showed that CAC1 was significantly associated with the probability of infection ($p = 0.07$) but not dam infection status ($p = 0.81$).

The logistic regression model was the following:

$$\text{Probability of infection in hutch} = \frac{\exp(-1.287 - 0.393*\text{CAC1})}{1 + \exp(-1.287 - 0.393*\text{CAC1})}$$

TABLE II. Information on Colostral Antibody Concentration (CAC) During the First Week of Life (CAC1), Dam Average Peripheral Lymphocyte Count per μL of Blood (PBLC) During Pregnancy, Age and CAC at Tests, Estimated Age at Infection and Probability of Infection While in a Hutch for 16 Calves for which a Negative Test Was Not Observed for 60 Days Following the Last Test with Lowest CAC (Status Uncertain Calves)

Calf #	CAC1	Dam Average PBLC	Estimated Age at Infection (days)	Probability of Infection While in a Hutch	Age at Test in Days (CAC at Test)			
203	4	27,170	44/100[a]	0.47/0.06[a]	106 (1)	184 (4)		
369	3	19,212	18/64	0.64/0.42	30 (2)	72 (0)	147 (3)	
377	3	4,819	22/70	0.71/0.52	57 (2)	78 (0)	155 (4)	
388	3	2,501	39/111	0.47/0.02	71 (1)	119 (0)	188 (4)	
393	2	5,128	30/59	0.61/0.47	33 (1)	53 (0)	67 (0)	170 (4)
397	4	3,338	45/100	0.59/0.15	117 (1)	200 (4)		
398	1-2	4,072	43/107	0.58/0.15	78 (1)	103 (0)	181 (4)	
428	4	11,800	51[b]	0.54[b]	69 (4)	135 (3)	205 (2)	212 (3)
788	4	15,900	61/126	0.30/0.01	138 (1)	233 (4)		
795	3	4,930	41/91	0.56/0.24	98 (1)	192 (4)		
804	4	N-INF[c]	35/96	0.60/0.21	71 (2)	103 (0)	181 (4)	
841	4	7,776	15/55	0.65/0.50	62 (1)	141 (4)		
869	4	7,270	9/95	1.00/1.00	83 (1)	97 (0)	112 (0)	128 (2)
871	3	N-INF	6/95	1.00/1.00	78 (1)	92 (0)	108 (0)	124 (4)
874	1-2	4,348	10/98	1.00/1.00	70 (1)	84 (0)	115 (0)	130 (2)
886	3	9,731	1/34	1.00/1.00	36 (1)	51 (0)	67 (1)	

[a]First method of estimation/second method of estimation (see text)
[b]For method of estimation, see text
[c]N-INF: noninfected dam

TABLE III. Proportion (%) of Calves that became Infected in Hutches by Categories of Maternal Bovine Leukemia Virus (BLV) Infection Status and Colostral BLV Antibody Concentration During the First Week of Life

Colostral Antibody Concentration	Dam		
	Infected	Not Infected	Total
Negative	3.72/16 (23%)	23.63/106 (22%)	27.35/122 (22%)
Low	2.76/30[a] (9%)	3.81/24 (16%)	6.57/54[a] (12%)
	2.19/30[b] (7%)		6.00/54[b] (11%)
High	6.95/52[a] (13%)	1.60/16[a] (10%)	8.55/68[a] (13%)
	4.74/52[b] (9%)	1.21/16[b] (8%)	5.95/68[b] (9%)
Total	13.43/98[a] (14%)	29.04/146[a] (20%)	42.47/244[a] (17%)
	10.65/98[b] (11%)	28.65/146[b] (20%)	39.30/244[b] (16%)

[a]Figures for first method of estimation of age at infection for the 15 SU calves
[b]Figures for second method of estimation of age at infection for the 15 SU calves

According to this model, calves that had a negative CAC1 had a greater probability of infection (0.22) compared to calves with a low CAC1 (0.16) or a high CAC1 (0.11). Relative risks of infection for calves with negative CAC1 were 1.38 (0.22/0.16) and 2.00 (0.22/0.11) compared to calves with low and high CAC1, respectively.

Using the second method to estimate age at infection in the 15 SU calves, the proportions of calves estimated to have become infected while housed in hutches decreased slightly (Table III). Results of logistic regression analysis showed that dam infection status was still not found to be associated with the probability of infection ($p = 0.68$). However, the strength of the association of the probability of infection with CAC1 was stronger ($p = 0.01$) than when the first estimation method was used. Regression coefficients were -1.271 (intercept) and -0.596 (CAC1). Relative risks of infection for calves with negative CAC1 were 1.69 (0.22/0.13) and 2.75 (0.22/0.08) compared to calves with low and high CAC1, respectively.

The Cox model was then used to ascertain the relationship between the most likely age at infection and the two factors, dam infection status and CAC. Because convergence was not obtained when concentrations were designated as 0, 1, 2, 3 and 4, three categories for CAC were used, namely negative, low and high. The age at which a CAC changed from high to low or from low to negative was designated as the age at which the CAC changed from 3 to 2 or from 1 to 0, respectively.

When the first method to estimate age at infection was used, dam infection status and CAC were not found to be associated with the estimated age at infection ($p = 0.52$ and $p = 0.88$, respectively). When the second method of estimation was used, dam infection status again was not found to be associated with time of infection ($p = 0.90$). High CAC, however, was found to be associated with a delay in time of infection ($p < 0.001$). The resulting model can be expressed as follows:

$$h[t,CAC(t)] = h0(t) \exp [-1.226*CAC(t)].$$

This model indicates that the risk of infection for a calf at time t, h[t,CAC(t)], is equal to the baseline risk of infection for any calf at that time, h0(t), increased or decreased depending on its specific CAC at time t, CAC(t). Calves with high CAC were 3.4 times less likely to become infected than calves with low CAC. Calves with low CAC were also 3.4 times less likely to become infected while in hutches than calves with negative CAC. Calves with high CAC were 11.6 times less likely to become infected than calves with negative CAC.

To estimate the extent of protection conferred through colostral BLV antibodies, five situations were simulated for calves with CAC1 of 0, 1, 2, 3 and 4. It was assumed that duration of stay at a given CAC was equal to the median duration of stay (10). For instance, if a calf with CAC1 of 3 had a high CAC for 12 days, a low CAC for 56 days and a negative CAC thereafter, it would be 11.6 times less likely

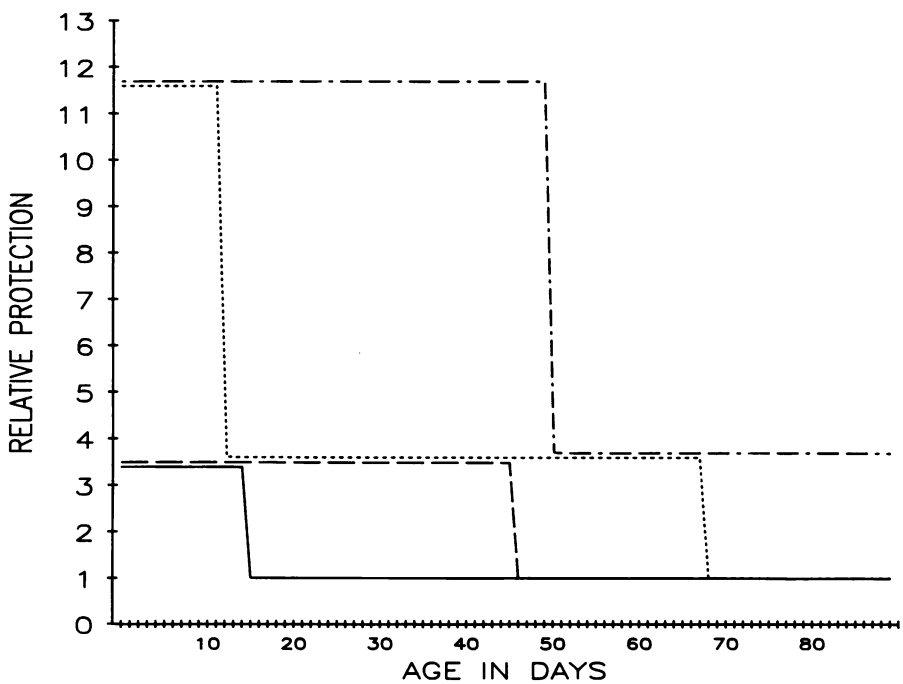

Fig. 2. Relative protection against BLV infection for calves with colostral antibody concentrations during the first week of life of 1 (—), 2 (— —), 3 (- - - -), and 4 (— · —) compared to calves with colostral antibody concentration of 0.

to become infected while in a hutch than a calf with a negative CAC for the first 12 days of life (Fig. 2). The presumed relative protection against infection would drop to 3.4 between 12 and 68 days of age and to 1.0 when CAC became negative (Fig. 2).

Percentages of infected calves with negative CAC1 and born to BLV-infected or noninfected cows were 23% and 22%, respectively (Table III). The estimated number of calves infected by the time they were removed from hutches was 3.72 (Table III). This number was derived from three calves that had a probability of being infected by the time they left hutches of 1.0 and from one calf with a probability of 0.72. The most likely ages at infection for the first three calves were birth, 43 and 48 days and, for the other calf, it was 93 days. Average dam PBLC during pregnancy was 13,178 cells/μL for the calf likely to have been infected at birth and 3,859, 3,880 and 7,166 for the other three calves.

DISCUSSION

The overall finding that more calves that did not receive BLV colostral antibodies became infected while housed in hutches, compared to calves that received BLV colostral antibodies, is similar to previous observations that BLV antibodies present in colostrum can prevent oral or intradermal infection (12,15,16), the oral route being an effective route during the first three days of life (12). Presence of high CAC, however, did not seem to completely protect calves against infection.

The extent to which colostral antibodies prevented infection was difficult to quantify. The Cox model was an appropriate model to address our objective, that is, to quantify the effect of CAC, a time-dependent factor, on the risk of infection. Individual variations in the decay of colostral antibodies for calves with the same CAC1, therefore, could be accounted for in the analysis. This permitted a better evaluation of the effect of CAC than when the logistic model was used because CAC, for the logistic model, had to be defined as fixed. This is one of the reasons why results of both analyses were different. However, because the Cox model considers time to infection, any errors in estimating day of infection would be expected to alter results of Cox model analyses more than those of logistic regression analyses. The latter method considered whether calves were infected by about 90 days of age. Both analyses helped, therefore, in addressing the objective. Results of logistic regression gave more evidence for a protective role of BLV colostral antibodies than those of the Cox model. Results of the Cox model analysis showed that a high CAC could protect calves from infection by up to 11.6 times more than calves with no detectable colostral BLV antibodies, the "true" value probably being less.

Calves that became infected while housed in hutches could have become infected orally during the first three days of life via infected milk or via infected colostrum, provided there was BLV in excess of neutralizing antibodies. Infection also could have occurred by intranasal exposure, that is by inhalation of BLV-infected lymphocytes present in milk, as indicated in previous studies in which the respiratory route was found to be an effective route of infection at any age (17,18). It is possible that iatrogenic transmission may have occurred through infected needles or contaminated tubes used for oral rehydration; however, the likelihood of these means of transmission may be low (3,19).

One calf classified as infected *in utero* or during the early *postpartum* period was born on July 30 from a cow that later developed malignant lymphoma. It was interesting to see that all calves born around that time (between July 28 and August 1), and followed-up until they left the hutch, became infected. Those calves were numbers 869, 871, 874 (Table II) and one calf that was born to a noninfected cow and did not receive colostral antibodies. Infection in these four calves may have occurred if they received colostrum and/or milk from the malignant lymphoma cow that contributed to pooled colostrum and milk during the time they were born.

The importance of milk as a source of infection under field conditions has been perceived to be minimal by some (2,3), but significant enough by others to prompt their expression of concern over feeding milk (4,5). The design and settings of these studies may have contributed to differences in opinions about milk-borne transmission. In the first two studies (2,3), the time at the first positive BLV serological result was used as the time of infection. Because of the lag in time from infection to seroconversion, using the time at first positive result as the time at infection would underestimate infection rates while calves were fed milk. In addition, colostrum-management could have been different from the herd in our study. In one study (3), most calves received large amounts of BLV colostral antibodies which conceivably could have prevented milk-borne infection more than in our study. In studies in which milk was suspected of being the source of infection in as many as 27% of calves (4), the possible transmission by physical contact was not explored. Failing to consider other means of transmission would likely result in the overestimation of transmission through infected milk.

The amount of virus shed in milk during lactation, and the factors influencing the amount of shedding and rate of shedding among cows, also may explain some of the differences in estimates of milk-borne transmission. One factor might be the cow's antigen-positive status. It was suggested that antigen-positive cows might be more likely to shed virus in the milk than antigen-negative cows (20) and, therefore, the infection status of the milking herd could influence risk of calf exposure to infected milk. Factors influencing the amount of shedding have not been investigated.

It also was observed that four of 16 calves born from infected dams and having a negative CAC1 became infected. One of these calves was born to a dam with high PBLC and could have become infected at birth. A possible mechanism of infection could be related to exposure to maternal blood at parturition (1). In one study, 10.3% (3/29) of calves were suspected to have been infected around calving (2), which is a greater proportion than that of 6.3% (1/16) estimated in the present study.

The design of our study, the management of the calves, and results of previous work on iatrogenic transmission (3,19), preclude major means of transmission other than milk and colostrum. Calves suspected to have been infected *in utero* or early *postpartum* were not included in analyses. The fact that calves were housed in individual hutches placed far enough apart to prevent calf-to-calf contact minimized transmission directly from an infected calf. Moreover, needle-borne transmission of BLV has been found to be insignificant or nonexistent (3,19). One possible way to differentiate further the role of colostrum and milk in BLV transmission would be to compare rates of infection in calves that had colostral antibodies and that were fed milk from noninfected cows to those for calves that did not have colostral antibodies and that were fed milk from infected or noninfected cows.

The practical application of results of the present study is that feeding colostrum with BLV antibodies may prevent as much as half of the infection taking place during the first three months [(22.4%-12.4%)/ 22.4% = 44.6%]. In a control program of BLV infection, colostrum-derived BLV antibodies, however, will delay early detection of infection in calves. If calves are fed colostrum from noninfected cows, the results of this study offer a basis for the recommendation of the feeding of powdered milk, milk replacer, and/or whole milk from noninfected cows to reduce calfhood infection.

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