

Role of Colostrum and Milk in the Natural Transmission of the Bovine Leukemia Virus¹

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ABSTRACT

The role of colostrum and milk in the transmission of the bovine leukemia virus (BLV) was examined by monitoring the development of BLV infection in calves that were fed since birth on colostrum and milk from their BLV-positive dams and then reared in complete or partial isolation from infected cattle. Twenty-one of the 25 calves raised in complete isolation remained negative for BLV until the last evaluation. At this time, 14 calves were older than 28 months of age, and seven were 16 to 20 months old. Three calves in this group became BLV positive before the age of five months, and one became so at the age of 12 months. Of the 16 calves raised in partial isolation, two were positive at the ages of 11 and 18 months, respectively. The other 14 calves remained negative during the 26 to 29 months of observation. It is not known if the six animals that became BLV positive in these two groups were infected by milk, or prenatally, or during contact with their infected dams. While only six of the 41 calves raised in isolation became infected, all 18 calves raised in contact with BLV-positive cows became infected before the age of 26 to 29 months of age, and 12 were positive at 16 to 20 months of age. Thus, it is apparent that, under natural conditions, milk-borne transmission of BLV, if it occurs at all, is much less frequent than contact transmission, despite the fact that, as shown in previous studies, infectious BLV is present in the colostrum and milk of most BLV-positive cows. Passively acquired maternal antibodies to BLV were probably responsible for the resistance of the calves to milk-borne infection.

INTRODUCTION

As the result of the research conducted during the past several years, it has become apparent that bovine lymphosarcoma and BLV⁴ infection provide a most valuable and, in some respects, unique model system for studies on the etiology, pathogenesis, and prophylaxis of leukemia in mammals (5, 6). Thus, elucidation of the natural mode of transmission of BLV is important not only for veterinary medicine but also for leukemia research in general.

There is no evidence that, in cattle, BLV infects cells other than the lymphocytes (1, 6). BLV-infected lymphocytes usually do not produce virus particles or express viral antigens *in vivo*,

but they begin to do so only after a few hr of *in vitro* cultivation (1, 6, 18). Viremia, *i.e.*, the presence of free BLV particles in blood, has not been convincingly demonstrated. On the basis of this information, it seems logical to assume that natural transmission of BLV results from the transfer of infected lymphocytes rather than BLV particles.

Studies on well-characterized cattle populations have shown that BLV is spread mainly by contact (2, 11, 15), but the mechanism by which this occurs has not yet been clarified. There is strong evidence that insect vectors are involved (2). BLV antigen has been detected in the urine of infected cattle (13), but the role of this and other secretions and excretions in the natural mode of transmission of BLV is unknown.

Less than 20% of the calves born to BLV-infected dams become infected before birth. Prenatal transmission seems to occur only through the placenta (16). The fact that BLV and its genome have been detected only in the lymphocytes of infected cattle (1, 6) argues strongly against the possibility that BLV is transmitted genetically.

The role of milk in the transmission of BLV has been the subject of much speculation. Infectious BLV and/or BLV-infected cells are present in colostrum and milk of most naturally infected cows (9). However, this does not necessarily mean that calves become infected with BLV by the oral route under natural conditions. Burny *et al.* (3) and Straub *et al.* (20) have concluded that bovine leukosis, and therefore BLV, is transmitted by milk. However, in none of the studies on which this conclusion was based were the calves tested for BLV infection or BLV antibodies before nursing. This makes it impossible to determine if the calves that subsequently developed BLV antibodies or persistent lymphocytosis (a benign condition that seems to be caused by BLV) were infected prenatally rather than by milk. Furthermore, in some of these studies, it is not clear if the calves were kept in strict isolation in order to avoid infection by contact. Thus, a critical assessment of these data fails to support the conclusion that milk-borne transmission of BLV does occur.

The present study was undertaken to evaluate the role of colostrum and milk in the natural transmission of BLV under controlled conditions. For this purpose, the development of BLV infection was monitored in cattle that were fed colostrum and milk from their BLV-infected dams since birth and then reared in isolation or in continuous contact with infected cattle. Some of the results of this study have been included in a preliminary report published elsewhere (10).

MATERIALS AND METHODS

RIA. The BLV gp was purified and radioiodinated to a specific activity of 3 to 4 × 10⁴ cpm/ng as described by Devare and Stephenson (4). The double-antibody precipitation assay using labeled BLV gp was conducted essentially as described by Strand and August (19). Each

¹ This work was supported by USPHS Grant 3 P01 CA 14193, a grant from the Wetterberg Foundation, and Pennsylvania Department of Agriculture Grant ME-24.

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⁴ The abbreviations used are: BLV, bovine leukemia virus; RIA, radioimmunoassay; gp, glycoprotein; CRIA, competitive radioimmunoassay; SIA, syncytia infectivity assay.

Received July 17, 1981; accepted September 4, 1981.

serum was tested at 2 dilutions: 1:5 and 1:10 (1:50 and 1:100 final concentration in the incubation mixture), respectively. Sera that bound 20% or more of precipitable antigen at any of these dilutions were considered positive for antibodies against BLV gp.

Preparation of Cell Extracts. Peripheral blood lymphocytes were isolated by hypotonic shock and cultured for 48 hr in the presence of phytohemagglutinin as described by Stock and Ferrer (18). Following incubation, the lymphocytes were washed once by centrifugation at $1000 \times g$ for 15 min. The cell pack was resuspended in buffer [0.02 M Tris:0.1 M NaCl:0.001 M EDTA (pH 7.5):0.5% Nonidet P-40:0.2% sodium deoxycholate:2 mM phenylmethylsulfonyl fluoride] to a final concentration of 30×10^6 lymphocytes per ml, frozen, and thawed 3 times. The extracts were then clarified by centrifugation at $1000 \times g$ for 15 min.

CRIA. Cell extracts were tested for the major internal BLV antigen (p25), applying the CRIA described by McDonald and Ferrer (14). An extract was considered positive in the CRIA if 40 μ l of it (equivalent to 10^6 lymphocytes) or less displaced 20% or more of the iodinated antigen to BLV p25 antigen.

SIA. This assay was conducted using either early passages of bovine embryonic spleen cells (8) or feline CC81 cells (7) as the indicator system. The SIA has proven to be a specific, reproducible, and sensitive method for the detection of infectious BLV in blood lymphocytes (12).

Animals and Experimental Design. The dams of all calves used in this study were naturally infected with BLV and belonged to a multiple-case Jersey dairy herd, known as the BF herd, kept since 1964 at the University of Pennsylvania School of Veterinary Medicine. Nearly all cattle older than 3 years of age in the BF herd are infected with BLV, but at birth, before the ingestion of colostrum, less than 20% of the BF calves are BLV positive (11, 16).

In the present study, BF calves were assigned to 3 experimental groups. The calves in the first group were separated from their dams within 9 to 14 hr after birth and then transferred to a holding facility where they were kept for 5 to 6 weeks. During this time, each calf received 8 to 14% of its body weight daily in fresh colostrum and milk from its infected dam. Beginning on the fourth day of life, the calves were also fed a commercial milk replacer. Subsequently, the calves were moved to an open-fronted pole shed located about 300 m from the barns and pasture holding BLV-positive cattle. Here, the calves were raised as a group and were attended by personnel who had no contact with BLV-infected animals. Vehicles, farm machinery, and utensils used in barns holding BLV-infected cattle were not allowed in the isolation facilities where these calves were raised.

The calves in the second group were kept with their BLV-infected dams for 4 to 6 weeks and then raised in partial isolation in an open-fronted barn which is 50 to 100 meters from the barns and pastures holding the BLV-infected cows. The calves in this group were attended by the herdsman in charge of the infected herd. No precautions were taken to avoid transportation of materials contaminated by excretions from infected cattle by personnel, farm machinery, utensils, etc., to the

facility where these calves were kept.

The calves of the third group were nursed on their BLV-positive dams for 4 to 6 weeks and then raised in the facilities holding the dry BF cows. Crown weaning rings were put in the nose of each calf at the age of 4 to 6 weeks to prevent suckling. Most of the calves were bred at approximately 1.5 years of age, and after parturition, they were transferred to the facilities holding the milking herd.

Each experimental group had approximately the same number of males and females. Bull calves were castrated at 4 to 5 months of age.

RESULTS

Table 1 summarizes the data obtained in this study. Before the ingestion of colostrum, all the calves in the 3 experimental groups were negative for BLV antibodies, as determined by the RIA gp, and negative for infectious BLV, as determined by the CRIA and/or the SIA. Following the ingestion of colostrum, all calves had BLV antibodies for 3 to 6 months. That these were passively acquired maternal antibodies is indicated by the fact that, by 7 to 9 months of age, most of the calves became antibody negative.

Four of the 25 calves in the group raised in complete isolation were positive in the CRIA and SIA at the ages of 1, 2, 5, and 12 months, respectively. These animals were removed from the isolation facility soon after the results of the assays were available. The remaining 21 calves were consistently negative in the RIA, CRIA, and SIA. At last evaluation, 7 of the animals were 16 to 20 months old, and 14 were older than 27 months of age.

The group raised in partial isolation originally consisted of 17 calves that, before nursing, were negative for BLV antibodies in the virus neutralization test (12). Subsequently, the precolostral serum samples from these calves were examined in the RIA gp, and one calf was found to be positive. Since this calf was probably infected prenatally, it was excluded from the experiment. One of the 16 calves in this group was found to be BLV positive at 44 weeks of age and was removed from the isolation facility. Of the remaining 15 calves, one became positive for virus and antibody by the age of 18 months. The other 14 calves were consistently negative in the RIA and SIA until the end of the isolation period. At this time, the animals were 26 to 29 months old. These 14 calves were subsequently introduced into the facilities holding the adult infected herd, and all became BLV positive within 1.5 years (data not shown).

None of the 18 calves in the group raised in continuous contact with infected cattle was tested in the SIA or CRIA

Table 1
Presence of BLV and BLV antibody in cattle raised on colostrum and milk from their BLV-infected dams and maintained either in isolation from or in continuous contact with BLV-infected cattle

Age	Cattle (positive/total) raised in					
	Complete isolation		Partial isolation		Continuous contact	
	V ^a	Ab	V	Ab	V	Ab
0 day (precolostrum)	0/25	0/25	0/16	0/16	0/18	0/18
1 wk-5 mos.	3 ^b /25	20/25	0/16	16/16	ND	18/18
7-9 mos.	0/22	0/22	0/16	0/16	4/18	4/18
11-14 mos.	1 ^b /22	1/22	1 ^b /16	1/16	8/18	8/18
16-20 mos.	0/21	0/21	1 ^b /15	1 ^b /15	12/18	12/18
25-34 mos.	0/14	0/14	0/14	0/14	18/18	18/18

^a V, virus as detected by the CRIA and/or SIA for BLV; Ab, BLV antibody as detected by the RIA gp; ND, not done.

^b These animals were removed from the isolation facilities within 1 week after the results of the tests were available.

during the 5-month period following the ingestion of colostrum. Four of these animals were positive in the RIA and SIA at 7 to 9 months of age. Eight additional animals in this group were positive by 20 months of age, and all 18 were positive by the age of 26 to 29 months. None of the animals in this group was removed from the facilities, and all continued to be positive for as long as they were tested.

DISCUSSION

Infectious BLV and/or BLV-infected cells are present in colostrum and milk from most BLV-positive cows (9). However, in the present study, BLV was detected in only 6 of the 41 cattle that ingested colostrum and milk from their BLV-infected dams since birth and were raised in partial or complete isolation. In contrast, all 18 control BLV calves that were raised in continuous contact with BLV-infected cattle were positive by 26 to 29 months of age.

Milk-borne transmission could account for the infected animals found in the isolated group. However, the 3 calves that became virus positive during their first 5 to 6 months of age could have been infected prenatally with small quantities of BLV or at the very end of the intrauterine life, thus escaping detection at birth. Infection of the calf that was positive at 11 months of age in the partially isolated group could have occurred during contact with the infected dams and remained latent for several months. Alternatively, this calf and the calf that was positive at 18 months of age could have become infected by virus transmitted by insect vectors or fomites from the BLV-infected cows that were maintained only 50 to 100 m away.

From these data, it seems that, despite the frequency with which infectious BLV is present in the colostrum and milk of infected cows, milk-borne transmission of BLV is infrequent, if in fact it occurs at all.

The resistance of calves to milk-borne infection can be attributed to the virus-neutralizing antibodies that, as shown in this and previous studies (12, 16), all calves nursed on BLV-positive dams acquire through colostrum and have in their serum for as long as 6 months. It is also possible that the infectivity of BLV particles and/or BLV-infected cells in colostrum and milk may be lost through contact with saliva and gastric and intestinal secretions or that they cannot pass across the gut wall. Indeed, it is well known that the intestinal absorption of macromolecules in cattle is limited to the first 24 to 36 hr of life (17). Furthermore, results of a preliminary experiment (21) suggest that the susceptibility of calves to BLV infection upon the oral administration of BLV-infected blood lymphocytes decreases drastically after the first days of life. Although this experiment suggested that BLV infection can be induced in calves by feeding them immediately after birth large numbers of lymphocytes, the high titers of viral neutralizing antibodies present in the colostrum of BLV-positive dams most likely block BLV and/or BLV-infected lymphocytes which may be present in this secretion.

Based upon these considerations, it is conceivable that conditions resulting in the massive passage of lymphocytes into colostrum and/or milk or that conditions that affect the integrity of the intestinal mucosa may increase the risk of milk-borne infection with BLV. Antibody titers in milk decrease very rapidly after the first day of lactation. Therefore, it is possible that

calves may become infected with BLV if, during the first hr of life, they are fed milk rather than colostrum from infected dams.

The resistance of calves to BLV infection during the first months of life is undoubtedly one of the explanations for the observation made in the BF herd and other dairy herds that the incidence of BLV infection is much higher in cattle older than 2 years of age than in younger animals (5, 16).

The information, that colostrum and milk do not play a primary role in the spread of BLV under natural conditions and that calves born to and nursed on BLV-infected dams may be protected against BLV infection during the first months of life by colostral antibodies, is important in the design of control and eradication programs. In this connection, it is also important to stress that physical separation by a relatively short distance may provide an effective barrier in preventing cattle from becoming infected with BLV. Indeed, all but 2 of the cattle kept 50 to 100 meters from a large group of BLV-infected cows for about 2 years remained BLV free. The distance required to prevent transmission of BLV from animal to animal probably depends in part upon the prevalence of potential blood-sucking vectors in the area. Biting flies are persistent feeders and usually return to the same animal or group of animals for completion of their blood meals. If necessary, however, some flies can travel long distances searching for a host.

In conclusion, the present study shows that milk-borne transmission, if it occurs at all, plays a secondary role in the natural spread of BLV. This study also extends previous evidence that BLV is readily transmitted by contact.

ACKNOWLEDGMENTS

The author wishes to thank Cheryl Zinn for technical assistance and Betty Thompson for typing the manuscript.

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Cancer Res 1981;41:4906-4909.

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