

# Seroepidemiological Evidence for Horizontal Transmission of Bovine C-type Virus<sup>1</sup>

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## SUMMARY

Thirty colostrum-deprived calves from leukemia-free herds were foster nursed for 10 weeks on cows infected with bovine C-type virus (BLV) from multiple-case herds or on cows from leukemia-free herds. After weaning, the calves were raised in continuous contact with BLV-infected animals of approximately the same ages. Sera collected at 6 to 18 and 43 to 48 months of age were examined for the presence of antibodies to BLV by the immunofluorescent antibody test.

At 6 to 18 months of age, only 1 of the 30 calves from leukemia-free herds had a detectable antibody response to BLV. By 43 to 48 months of age the number of antibody-positive animals had risen to 17. The foster dam's herd of origin did not significantly affect the rate of BLV infection.

These results indicate that BLV can be horizontally transmitted from infected to noninfected animals.

## INTRODUCTION

Seroepidemiological studies have provided strong evidence supporting the etiological role of BLV<sup>3</sup> in leukemia of cattle (4-8, 20). In addition, transmission experiments in sheep indicate that BLV is a leukemogenic virus (21).

Most cattle naturally infected with BLV possess antibodies to the major internal virion antigen. The detection of these antibodies by the IFA test on acetone-fixed cells provides an accurate and specific means of identifying cattle infected with BLV (4, 5, 7, 8). The immunodiffusion technique is also useful in detecting antibodies to the same BLV antigen (3-5, 7, 18). However, this technique is much less sensitive than the IFA test and is therefore inadequate for ruling out BLV infection (4, 8). More recently, the complement fixation test has also been applied to detect antibodies to BLV (19).

Studies were initiated to determine the natural mode of transmission of BLV as a possible basis for a bovine leukemia eradication program. At birth, most calves in a

multiple-case herd are not infected with BLV and do not have BLV antibodies. However, the majority of these animals eventually become infected with BLV and develop detectable antibodies to the virus (unpublished observations). Thus, it appears likely that natural infection with BLV takes place either through milk or by horizontal transmission later in life. In order to test these possibilities more critically, colostrum-deprived calves from leukemia-free herds were foster nursed on cows from multiple-case herds or from leukemia-free herds. The calves were then raised in contact with BLV-infected animals and tested for BLV infection by the IFA test.

## MATERIALS AND METHODS

**Immunofluorescent Antibody Test.** Preparation of the BLV-infected target cells, the fluorescent staining procedure, and the specificity of the IFA test have been described (3, 4, 6). Briefly, samples of a bovine cell line, NBC-13 (9, 12), in which 15 to 20% of the cells contain viral BLV antigen, were added to the wells of special slides prepared according to the procedure of Hirshaut *et al.* (13). The cells were then fixed in acetone and 0.05 ml of the serum to be tested was placed in one of the wells. The same amount of reference BLV Serum 27-125 (3, 6) was added to another well as a positive control. Serum from a cow in a leukemia-free, BLV-free herd was added to another well as a negative control, and a conjugate control well received phosphate-buffered saline (0.01 M sodium phosphate-0.015 M NaCl solution, pH 7.2). The slides were then incubated at 37° for 45 min in a humidified chamber and washed, and fluorescein isothiocyanate-conjugated goat anti-bovine 7S  $\gamma$ -globulin was added to each well. After incubation and washing, the slides were mounted with a coverslip using a 50% glycerin solution and viewed with a fluorescent microscope.

**Animals.** Thirty colostrum-deprived calves were obtained over a 16-month period from leukemia-free Guernsey Herds BH and BI. These herds have been under continuous surveillance for the last 13 years (1, 4, 17). None of the 32 cattle tested in the 35-animal BH herd and only 6 of the 151 BI animals tested were reactive in the IFA test.

The calves were nursed for 10 weeks on foster dams from leukemia-free herds or from multiple-case herds. After weaning, the calves were raised in continuous contact with

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<sup>3</sup> The abbreviations used are: BLV, bovine C-type virus; IFA, immunofluorescent antibody; NBC, New Bolton Center.

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BLV-infected calves from multiple-case herds. The data on the level of BLV infection of the group of calves from the multiple-case herds are given in "Results."

Sera were collected from the calves during the 6- to 18-month and 43- to 48-month age periods, stored at  $-70^{\circ}$ , and tested for antibodies to BLV by the IFA test.

Of the 15 foster dams of multiple-case herd origin, 14 were from Jersey Herd BF. Fifty-six cases of leukemia have been documented in the BF herd during an 18-year period. Approximately 60% of cattle in this herd have persistent lymphocytosis, and at least 90% of the adult animals are infected with BLV. One foster dam, used to nurse 2 calves, came from multiple-case Jersey Herd GD which closely parallels the characteristics of the BF herd (1, 4, 17).

## RESULTS

Eight of the 30 calves from leukemia-free herds were foster nursed on 5 dams originating in leukemia-free study herds. Unfortunately, sera were obtained from only 2 of these 5 dams. The 2 dams that were tested and found to be negative for antibodies to BLV by the IFA test were used to nurse 4 of the 8 calves in this group. Since BLV infection is either absent or rare in leukemia-free herds, it is highly probable that the 3 untested dams in this group were also negative. The remaining 22 calves were foster nursed on 15 dams of multiple-case herd origin. The sera from 14 of these dams were tested, and 13 were found to be reactive in the IFA test.

After 6 to 18 months of exposure to BLV-infected animals, only 1 of the calves from the leukemia-free herds had antibodies to BLV (Table 1). This calf had been foster nursed on a BLV-infected dam from the BF herd. From the available data it cannot be determined whether the calf was infected during or after association with its foster dam. By 43 to 48 months of age, 16 additional calves from the leukemia-free herds had become reactors, bringing the total number of BLV antibody-positive animals to 17 (57%). A comparison of the data for the 2 groups of calves at each time interval suggests that the foster dam's herd of origin was not a significant factor in the development of antibodies to BLV (Table 1). Seventeen of the 77 calves (22%) derived

from multiple-case herds and living with the 30 calves from the leukemia-free herds were infected with BLV by 6 to 18 months of age. At 43 to 48 months of age, this number had increased to 47 (61%).

## DISCUSSION

It has been postulated that cells of all vertebrates contain repressed genomes of C-type tumor viruses that are vertically transmitted and may be activated by a variety of factors (15). It is unlikely that the antibodies to BLV detected in the calves from leukemia-free herds in the present experiment were formed in response to virus or viral antigens produced by activation of a repressed viral genome. Since BLV infection is either absent or rare in leukemia-free herds, it is reasonable to assume that, had these calves remained in their herds of origin, they would not have developed antibodies to BLV. In addition, our attempts to demonstrate a C-type virus genome in cells of cattle from leukemia-free herds have been unsuccessful. Therefore, it is apparent that the calves acquired BLV infection during the 40 to 48 months of having lived with infected animals.

Dutcher *et al.* (2) have reported the presence of structures resembling C-type virus particles in milk from the BF herd. Therefore, it is conceivable that milk might have been the source of BLV infection for calves foster nursed on BF dams. However, our data are inconsistent with this possibility since we found only 1 animal with detectable antibodies to BLV at 6 to 18 months of age in the group foster nursed on BF dams. In addition, at 43 to 48 months of age, the incidence of antibody-positive calves nursed on dams from leukemia-free herds was similar to the incidence of antibody-positive calves nursed on BLV-infected dams. Thus, it seems reasonable to conclude that the calves from leukemia-free herds acquired BLV by horizontal spread from the infected animals with which they were housed.

The present data do not eliminate the possibility of BLV transmission from parents to progeny either vertically or through milk. Both horizontal and vertical transmission has been demonstrated in the murine and feline leukemia virus systems. In murine leukemia, vertical transmission predominates (10, 14); in feline leukemia, horizontal transmission is more common (11, 16).

We have found that, in the BF herd, where at least 90% of the adult cattle are infected with BLV, only a few animals show the virus at birth (unpublished data). These observations are consistent with the idea that horizontal transmission is the most prevalent mode of transmission of BLV but that vertical transmission of this virus does occur occasionally.

The observation that natural transmission of BLV can occur horizontally suggests that control and eventually eradication of bovine leukemia from a herd may be accomplished by the separation or culling of infected animals.

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Table 1

Prevalence of antibody<sup>a</sup> to the internal BLV antigen in cattle from leukemia-free herds<sup>b</sup> following foster-nursing and continuous living with BLV-infected animals

Origin of foster dam	Antibody-positive animals at 6-18 mo. of age	Antibody-positive animals at 43-48 mo. of age
Leukemia-free herds	0/8 (0) <sup>c</sup>	5/8 (62.5)
Multiple-case herds <sup>d</sup>	1/22 (4.5)	12/22 (54.5)

<sup>a</sup> Serum tested by the indirect immunofluorescent test on acetone-fixed NBC-13 cell preparations having 15 to 20% positive cells when reacted with reference BLV serum.

<sup>b</sup> Animals from Herds BH and BI.

<sup>c</sup> Positive/total (percentage).

<sup>d</sup> Fourteen animals from multiple-case Herd BF and 1 from multiple-case Herd GD.

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