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AN EVALUATION
OF THE ROLE OF MILK IN THE NATURAL TRANSMISSION OF BLV

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Introduction.

The bovine leukemia virus (BLV) can be detected consistently in peripheral blood lymphocytes of infected cattle by electron microscopy (Miller et al., 1969; Ferrer et al., 1971; Stock and Ferrer, 1972), serological techniques (Ferrer et al., 1972; McDonald and Ferrer, 1976) and by the syncytia induction assay (Ferrer and Diglio, 1976; Diglio et al., 1978; Ferrer et al., 1976a). There is no evidence in cattle that BLV infects cells other than lymphocytes. To understand the natural history of BLV infection, including mode of transmission, it is necessary to bear in mind that BLV-infected lymphocytes usually do not produce virus particles or express viral antigens in vivo; indeed, BLV and BLV antigens can be detected only after the lymphocytes are cultivated in vitro (Stock and Ferrer, 1972; Baliga and Ferrer, 1977). Viremia, the presence of free BLV particles in the peripheral circulation, has not been demonstrated in infected cattle. These findings can be explained, at least in part, by the fact that virus-neutralizing antibodies are present in virtually all infected cattle (Ferrer et al., 1977).

It has been demonstrated that the large majority of cattle become infected with BLV by contact and that prenatal (vertical) infection is relatively infrequent (Piper et al., 1975; Ferrer et al., 1976b; Piper et al., 1979). Contact transmission of BLV occurs most readily during the summer months (Bech-Nielsen et al., 1978). This observation and the successful
recovery of BLV-infected bovine lymphocytes from tabanids allowed to feed on an infected cow (Bech-Nielsen et al., 1978) strongly suggest that biting insects play a major role in the spread of the virus. Since BLV particles are not usually produced in vivo, it seems logical to conclude that infection results from the transfer of lymphocytes rather than free BLV particles.

The role of milk in the transmission of BLV has been the subject of much speculation. However, there are no published results showing that cattle become infected by milk under natural conditions. In view of the apparent ease of BLV transmission by contact, it is evident that the isolation of recipient cattle is a fundamental requirement in experiments designed to critically determine if milk-borne transmission of BLV does occur.

In the present study, a group of cattle born to, and nursed, on BLV-positive dams were raised either in isolation or in continuous contact with infected animals and examined for the presence of the virus at various ages. The results indicate that as compared with contact transmission, milk-borne transmission, if it occurs, plays a secondary role in the spread of BLV under natural conditions.

Materials and methods.

**Cattle.** All cattle were from the multiple-case study herd BF maintained at the University of Pennsylvania School of Veterinary Medicine. A total of 69 histologically confirmed cases of lymphosarcoma have been documented in this herd during a 20 year period in a population at risk of 350 cattle (Abt et al., 1976; Ferrer et al., 1974). Virtually all adult BF animals are infected with BLV. The rate of prenatal infection with BLV in the BF herd is 18% (Abt et al., 1976; Ferrer et al., 1974; Piper et al., 1978).

**Serum and Colostrum.** Serum samples were collected from the jugular vein and stored at -70 °C. Colostrum samples were mixed with an equal volume of 0.15 M EDTA pH 7.5 and centrifuged at 3,000 × g for 15 minutes at 4 °C. The clear interphase formed between the lipid and the precipitated casein layers was removed and stored at -70 °C.

Reference serum Se-362, obtained from a cow with persistent lymphocytosis in the BF herd, has BLV neutralizing antibodies as well as antibodies to the major internal BLV antigen. This serum is free of detectable antibodies to other known bovine syncytial viruses, including the ubiquitous foamy-like bovine syncytial virus (BSV). Negative control serum Se-354, collected from a cow in a leukemia-free herd, is negative for antibodies to BLV, but has a high titer of antibodies to BSV. Details of the properties of these sera have been published (Diglio and Ferrer, 1976).

**Syncytia Infectivity Assay.** The syncytia infectivity assay (SIA) using viable peripheral blood lymphocytes as the inoculum is a highly sensitive and specific method for the detection of infectious BLV in cattle (Ferrer and Diglio, 1976; Ferrer et al., 1977). The assay was conducted as described in previous publications (Ferrer and Diglio, 1976; Ferrer et al., 1977; Diglio et al., 1978).

**Virus Neutralization Antibody (VNA) Test.** The BLV inoculum was the cell-free supernatant fluid from cell line BLV-bat2 cl4. This culture is free of mycoplasma as well as other common bovine viruses (Graves and Ferrer, 1976; Diglio et al., 1978). One ml of the supernatant fluid was mixed with an equal volume of either medium, reference serum Se-362 (positive control), control serum Se-354 (negative control), or test serum. Precolostrum serum samples were concentrated 5 times by lyophilization. All serum and colostrum samples were heat-inactivated (56 °C, 30 min) immediately before testing. After incubation at room temperature for 1 hour, the mixtures were tested in duplicate for syncytia-inducing activity. Further details of the VNA test have been reported (Ferrer et al., 1976a; Ferrer and Diglio, 1976). The VNA test has been shown to be one of the most sensitive serological methods for the in vivo diagnosis of BLV infection (Ferrer et al., 1977).

Results.

In this study, 34 BF calves that were BLV-free at birth, as determined by both the SIA and the VNA test, were allowed to nurse on their dams for 5-6 weeks. All the dams were positive for infectious BLV in the SIA and had virus neutralizing antibodies in both serum and colostrum. After weaning, 17 of the calves (Group A) were raised in isolation from infected cattle until the age of 25-29 months. The other 17 calves (Group B) were maintained in continuous contact with BLV-positive cattle for the same period of time. The assignment of
calves to the two groups was done in a random fashion. Each group had approximately the same number of males and females. Bull calves were castrated at 4-5 months of age.

The cumulative incidences of virus-positive and antibody-positive cattle in Groups A and B are shown in Figures 1 and 2, respectively. All calves were virus-negative and antibody-negative at birth before the ingestion of colostrum. At 1 and 3 Months of age, all calves in both groups became antibody-positive. Evidently, this antibody was passively acquired maternal antibody since none of the calves were infected at these ages. Moreover, the antibody was transient since at 6 months of age only 1 calf in group A and 2 calves in group B were antibody-positive. These 3 antibody positive animals were infected.

Only 3 of the 17 cattle in Group A became infected with BLV during the 25-29 month observation period (at the ages of 6, 12 and 18 months respectively). These animals were removed from the isolation area immediately after they were shown to be positive. In contrast, 7 of the 17 cattle (Group B) raised in contact with infected animals, were positive by 12 months of age and all 17 were positive by 25-29 months of age. As in previous studies (Ferrer et al., 1977), there was a close correlation between the presence and absence of the virus and the presence and absence of virus neutralizing antibody after the age of 6 months, as well as before the ingestion of colostrum.

Discussion.

In this study, BLV was detected in 3 of 17 cattle that were reared in isolation for over 2 years after they were weaned from their infected dams. In contrast, all 17 control cattle raised in contact with BLV-positive animals became infected during the same period of time.

Milk-borne transmission could account for the 3 positive animals found in the isolated group. However, it is also possible that 2 of these animals became infected through contact with the calf that was positive at the age of 6 months. Alternatively the 3 animals could have become infected by contact with their infected dams. Also, these animals could have been infected in utero with low doses of virus, so that they became positive in the SIA and VNA tests only several months after birth.

Preliminary data strongly suggest that BLV or BLV-infected lymphocytes are present in milk and colostrum of infected dams (Miller and Van der Maaten, in press; Kenyon S., and Ferrer J.F., Manuscript submitted for publication). However, as shown by our data, milk-borne transmission of BLV if it occurs, is rare. It seems evident that passively acquired maternal antibodies are responsible for the observed resistance of calves to BLV infection. Indeed, as shown by this and previous (Ferrer et al., 1977) studies, virtually all BLV-infected dams transmit virus-neutralizing antibodies to their calves via colostrum, and these antibodies persist in the calves for at least 3-5 months.

![Fig. 1](image1.png) Cumulative incidence of virus-positive (O) and antibody-positive (Δ) animals in a group (group A) of 17 calves born to, and nursed on, BLV-infected dams. The calves were raised in isolation after nursing for 5-6 weeks.

![Fig. 2](image2.png) Cumulative incidence of virus-positive (O) and antibody-positive (Δ) animals in a group (group B) of 17 calves born to, and nursed on, BLV-infected dams. The calves were raised in contact with BLV-infected cattle after nursing for 5-6 weeks.
months. It is well known that the intestinal absorption of macromolecules in cattle is limited to the first 24-36 hours of life (Shultz, 1973). Thus, it is probable that after this time BLV or BLV-infected lymphocytes cannot pass across the gut wall. Preliminary results supporting this possibility have been obtained in a study (Van der Maaten and Miller, 1978) in which large numbers of BLV-infected lymphocytes were administered orally to calves at various times after birth. BLV infection could be experimentally induced by the oral administration of large numbers of isolated infected lymphocytes during the first hours of life. However, under natural conditions, the high titers of virus neutralizing antibodies present in the colostrum of infected dams most likely block BLV and/or BLV-infected lymphocytes that may be released into this secretion.

It is conceivable that conditions resulting in a massive passage of lymphocytes into the colostrum and/or milk, or 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 3rd day of lactation. Therefore, it is possible that calves may become infected with BLV if, during the first hours of life, they are fed milk rather than colostrum collected from infected dams.

In conclusion, the present study shows that under natural conditions, milk does not play an important role in the spread of BLV. The data confirm and extend previous results (Piper et al., 1975; Ferrer et al., 1976b; Bech-Nielsen et al., 1978) demonstrating that BLV is readily transmitted by contact. This information is obviously of fundamental importance in designing eradication and control programs. Since in the large majority of cases BLV infection is the result of contact transmission, a vaccine against BLV would seem to be of great potential value.

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Summary

In order to evaluate the role of milk in the transmission of BLV, the presence of the virus and viral antibodies was investigated in cattle that were born to, and nursed on, infected dams and then raised in isolation or in contact with infected animals. Only 3 of the 17 isolated cattle became infected during 25-29 months of observation. During the same period of time, all 17 cattle raised in contact with BLV-positive animals developed BLV infection. From these results, it is apparent that milk-borne transmission of BLV, if it occurs, is much less frequent than contact transmission.

References


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