Progress for Control of Bovine Leukosis

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Bovine leukosis (the term as used will include lymphosarcoma, the related lymphocytosis, and subclinical infection with leukosis virus) has been studied intensively in recent years and a brief review for veterinarians is presented. Serological methods have now been applied to indicate and control bovine leukosis virus (BLV) infection. Since 1975, the Commission of European Communities (CEC), has sponsored international conferences annually (1, 2, 3, 4). These have enabled coordination and exchange of current results among the concerned laboratories in Europe, as well as Canada and the United States. Emphasis has been given to the adult, sometimes called enzootic, form of bovine leukosis both because of its economic significance as the most commonly occurring, and because it is the only form with which BLV has thus far been associated. In adult lymphosarcoma there may be only scattered lymph nodes enlarged (Figure 1), but the heart, abomasum and uterus often are involved.

Three other forms are grouped with the term sporadic bovine luekosis and neither BLV or BLV antibodies can be demonstrated. The infrequent juvenile, or calf, form may be fully developed at birth or delayed until the age of about two years. Visceral organs as well as nearly all lymph nodes (Figure 2) are usually affected. The thymic form is rare and manifested in cattle about two years old by lymphosarcoma extending from the anterior chest to the larynx (Figure 3). The cutaneous form is also rare (Figure 4). It occurs in cattle one and one-half to three years old. The scattered, discrete lesions resemble warts, but are congested and often exude serum. Lymph nodes later may become enlarged, as in the adult form. Spontaneous regression of tumors may occur in the adult, juvenile and cutaneous forms (5).

Bovine Leukosis Virus and Serological Tests

BLV particles have not been found in tissue or blood of infected cattle unless the cells are washed and cultured usually with a mitogen. They then produce virus (6). Possibly this is because antibodies to the viral glycoproteins (gp) in extracellular fluids inhibit maturation and release of virions from infected cells (7). The virus need not be released extracellularly, however, to spread infection. Infection can be passed by cells and from cell to cell through cellular division. Thus, BLV can establish a persisting infection inside cells that co-exists with specific antibody in body fluids. BLV first was visualized in 1968 when lymphocytes from cows with bovine leukosis were cultured *in vitro* with a mitogen and examined by electron microscopy. A peculiarity is that while lymphocytes from some cattle need a mitogen for virus production, lymphocytes from other cattle do not require mitogen and the presence of mitogen actually suppresses virus production by lymphocytes from a few animals (8). BLV contains two classes of antigens (9). The larger glycoprotein (gp) antigens are located on the outer coat of the virion and a smaller, protein antigen (p24) of 24,000 molecular weight is at the core of the virus particle. The antigens presently in use for detection of BLV antibodies contain principally, but not exclusively, gp antigens. Antibodies to BLV can be recognized by various serological tests. An agar gel immunodiffusion (AGID) test has been used widely and when done with care following approved procedures has given comparable results in different laboratories. The AGID test was recommended by the CEC conference of 1977 (3). A persistently BLV-infected tissue culture cell line originally derived from fetal lamb kidney cells is used for commercial production of AGID antigens (10).

BLV also can be demonstrated by polykaryocytosis, or syncytium formation in certain cell cultures after inoculation with infected material (10, 12, 13).

Only a few BLV infected cattle develop clinical lymphosarcoma. Three multiple case herds were studied for several years (14). In herd #1 of Table I there were 6 cases during 5 years when there was an average of 35 BLV infected cows per year, thus, with an average of 1.2 cases per year, 3.4% of the BLV infected cows developed tumor. In herd #2 there were 3 cases during 3 years when there was an average of 14.7 BLV infected cows, thus, 6.8% of BLV infected cows developed tumor. These relatively high rates of tumor stopped abruptly in both herds for no apparent reason. In Minnesota a 40 cow herd had 22 cases during a 10 year period, a tumor rate of 5.5% (personal communication from Dr. Dale Sorenson). In most herds the tumor rate is less. For example, in herd #3 (Table I) there were only 5 cases during 32 years among a yearly average of 220 cows. Adult lymphosarcoma, also called enzootic bovine leukosis, usually occurs in cattle more than three years old. Only some of these may have a persisting lymphocytosis preceding or with the tumor. Clinical lymphosarcoma was delayed ten years in a steer experimentally infected with BLV when only a few days old.

Serological data indicate natural BLV infection occurs essentially only in cattle (15). Of the four forms of clinical

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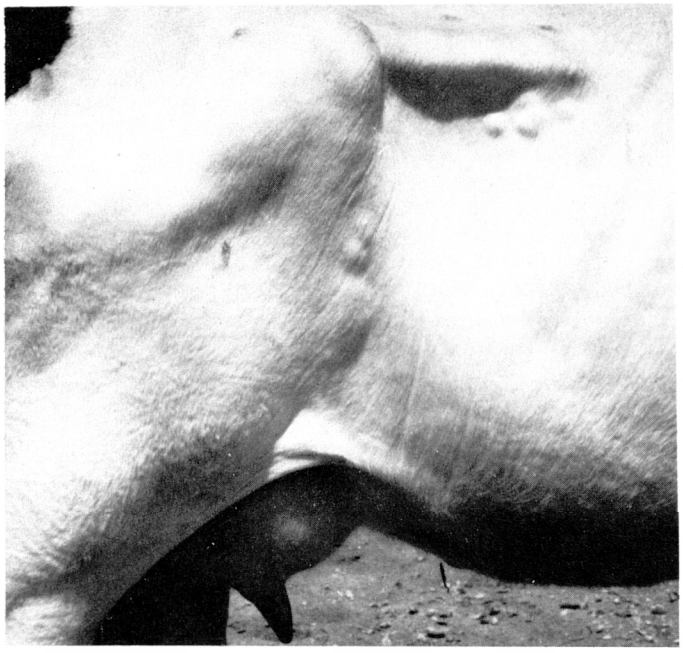

Figure 1. Enlarged prefemoral lymph node and hemal nodes of adult form of lymphosarcoma. This form is the only one associated with BLV infection. The term enzootic bovine leukosis has come to include cattle with BLV infection and no tumor as well as the adult tumor form.

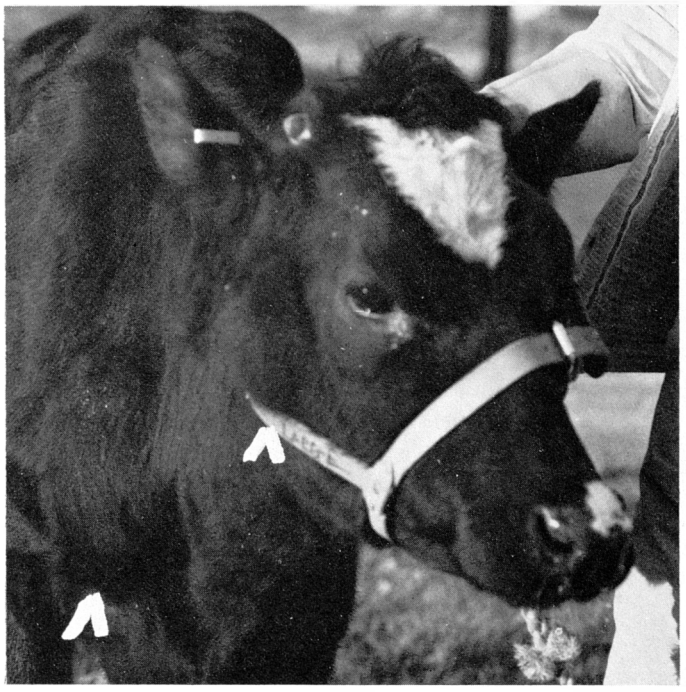

Figure 2. Juvenile form of bovine leukosis of an 8 month old calf. Marked enlargement of prescapular and parotid lymph nodes from lymphosarcoma is evident and other lymph nodes were similarly enlarged. BLV cannot be cultured from such cases and there are no antibodies to BLV. The dams of such calves are usually negative for BLV infection.

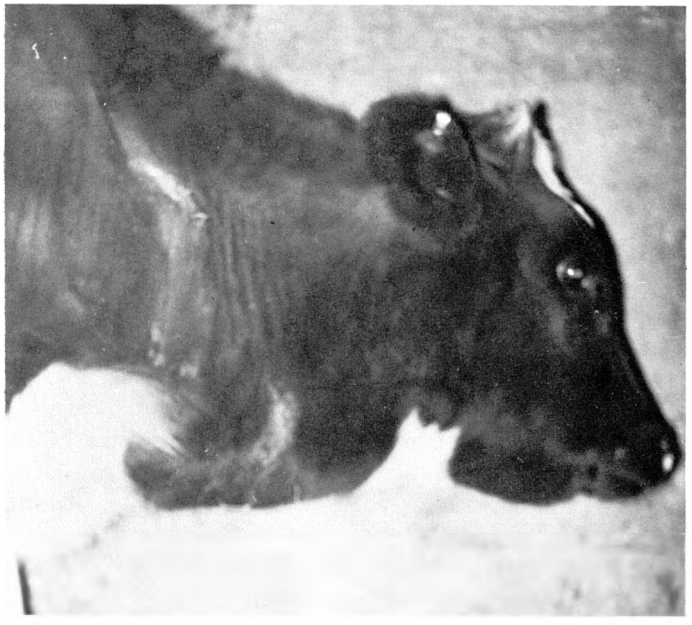

Figure 3. Thymic form of bovine leukosis with lymphosarcoma in region of neck previously occupied by normal thymus gland. Neither BLV or BLV antibodies have been found with this form of bovine leukosis.

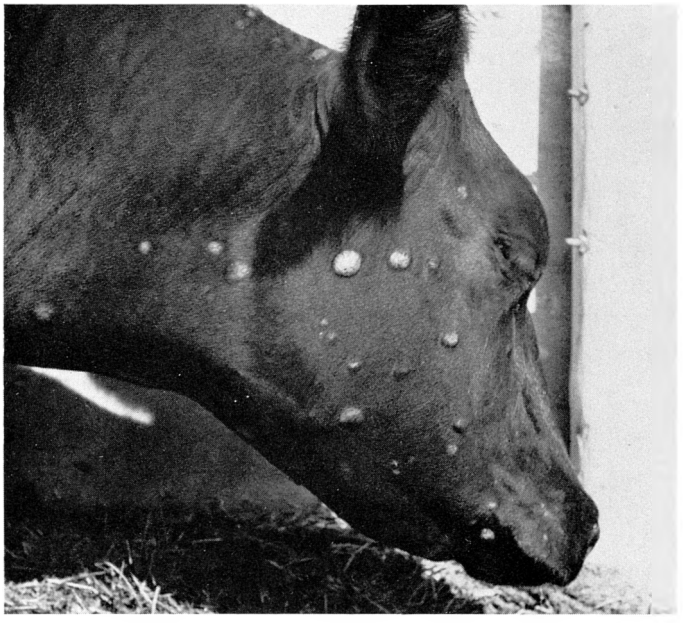

Figure 4. Cutaneous and skin form of bovine leukosis in a 2 1/2 year old cow. These lymphosarcoma lesions had regressed 3 months later. There was no recurrence or other signs of lymphosarcoma up to and including necropsy 12 1/2 years later.

lymphosarcoma the virus has been demonstrated only in material from cattle with adult lymphosarcoma, but sheep experimentally infected with BLV may develop any of the four forms of disease (16). The ovine tumors develop from one to six years after inoculation. There is no evidence of spread of BLV from experimentally infected sheep. The goat is the only other species of domestic animal known to be readily susceptible to BLV. They develop BLV antibodies and the virus can be recovered from inoculated goats, but none have developed tumors. Inoculation of BLV into chimpanzees induced antiviral antibody. Although serum antibody titers were persistent, virus could not be recovered and neoplasia has not been detected (17). **There is considerable evidence that BLV does not infect man (18).**

Extent of Bovine Leukosis

The infectious nature of bovine leukosis had been suspected for many years. Bendixen (19) has documented its spread from the area of what was then Lithuania, East Prussia and northern Poland to East Germany and Upper Saxony prior to, during, and following World War II. Before development of serological tests to indicate infection with BLV the estimates of prevalence of bovine leukosis were based on the occurrence of lymphocytosis or neoplasia. The incidence of bovine leukosis as seen in abattoirs in the eastern European areas in the early 1930's ranged from 160 to 600 per 100,000 cattle. Stahl estimated a rate of 100 to 300 per 100,000 cattle on farms in East Germany for 1955-56. In Denmark, Bendixen estimated a rate of 5.4 per 100,000 cattle on farms during 1953 to 1964, prior to initiation of the Danish program of eliminating herds where "leukosis" was diagnosed. This program reduced, but did not eliminate the disease. Later in Denmark today, herds with a low level of reactors to BLV antigen still existed and serological methods were expected to replace the surveillance by blood lymphocyte counts (4).

The data of the U.S.D.A. Meat Inspection Service on lymphoma (malignant lymphoma, lymphosarcoma) in slaughter cattle provides information on the increase of the disease. From 1917 to 1925 the reported rate varied between 4 and 6/100,000; 1938 14/100,000; 1950 to 1953 10/100,000; 1958 18/100,000; and 1964 17/100,000 (Baumgartener *et al,* 20), Feldman (21) used similar data for the years 1926 to 1929 to calculate a rate of 11.1/100,000, and Reissinger (22) found a rate of 21/100,000 for 1963, but he also noted a range of 3 to 95/100,000 at different slaughter plants, indicating geographic variation. These figures include tumors occuring in all ages of cattle. In 1977 the rate of condemnation in adult cattle was 21/100,000. Thus, regardless of probable variations in recording of tumors there is evidence of a slow increase of bovine leukosis in the U.S. Estimates for rate of occurrence of bovine leukisis in herds of dairy and beef cattle have been made. Perman (23), reported the leukosis rate in Minnesota to be 12/100,000 dairy cattle and 3.1/100,000 beef cattle; Theilen (24), 1964, found the overall rate in California to be 8/100,000, but in twenty-eight herds the incidence was 300/100,000 and in ten herds 120/100,000. In Michigan for the period of 1962-65 Connor *et al* (25) reported 19 cases/100,000 Holstein cows. The incidence in cows eight years of age and older was 36 per 100,000.

While the surveys for BLV antibodies conducted in the U.S. have been on rather haphazardly collected samples, they indicate infection is widespread. In an early serological survey of 4,394 cattle in north central states using the p24 antigen (ether treated) which now is know to be less sensitive than one containing BLV glycoprotein as well, there were reactors in 66 of 100 dairy herds and 7 of 50 beef herds (Baumgartener *et al,* 20). In the affected dairy herds the incidence of reactors varied from 2.1 to 44%, with an average of 10.2% for all cattle. In another group of states, House *et al* (26), tested samples from 1,295 beef and dairy cattle in seventy-four herds and found precipitating antibody to BLV glycoprotein in 28.2% of dairy catle and 2.6% of beef cattle.

A sensitive radio-immunoprecipitate assay for antibodies to both p24 and glycoprotein BLV antigens was applied to about 6,000 randomly selected bovine serum samples obtained from regional USDA brucella laboratories in 22

Table I. Yearly Rate of BLV Reactors in 3 Herds

Herd		1972	'73	'74	'75	'76	'77	'78	Total
# 1	Number tested	95	55	61	59	52	61	53	436
	% Reactors*	54	42	67	40	71	87	79	63
# 2	Number tested	ND	19	27	53	73	57	48	277
	% Reactors		53	48	41	56	72	71	57
# 3	Number tested	273	165	276	249	204	209	255	1631
	% Reactors	24	30	24	31	31	31	22	27

*Average percentage rate of reactors

Herd # 1 had 6 cows with lymphosarcoma in 1972 to 1976.
Herd # 2 had 4 cows with lymphosarcoma in 1972 to 1975.
Herd # 3 had 3 cows with lymphosarcoma 31 and 32 years ago, had 1 case 15 years ago and last case 4 years ago.

Figure 1. The bovine viral disease iceberg schematically portrays clinical disease as a small visible fraction of the total virus-host-environment interaction.

states (Devare and Stephenson, 27). Reactors in these states ranged from 2 to 41%. In one-third of the states 20% of the animals were positive. The high rates were not due to a small number of high incidence herds. In the state with the highest rate (41%), the 480 positive serum samples were from forty-three of a total eighty-two counties. Thus, it can be said that over half (perhaps three-fourths) of the dairy herds serologically surveyed in different parts of the U.S. have BLV infection involving from one or two animals to 80% of the herd.

Spread of Bovine Leukosis

It had been thought that spread of bovine leukosis was largely vertical from dam to progeny, that is, followed so-called blood lines implicating both infectious and genetic factors. European eradication programs, such as that begun in Denmark in 1965, required quarantine or slaughter of entire herds. Even though there may be genetic factors affecting susceptibility to BLV infection or tumor induction, prevention of BLV infection would eliminate the disease.

Investigation of BLV-infected bulls indicated they did not transmit the infection to progeny when used by artificial insemination and there was no contact of the bulls with the cows (28). Susceptible sheep inoculated with semen from infected bulls did not develop BLV infection (29). Although it has not been possible to extract nucleic acids from sperm in order to test for the BLV genome by hybridization, testicular tissue from a BLV infected bull was negative when it was tested by this technique (unpublished data, R. Callahan and C. Olson). Thus there is no evidence of vertical transmission of BLV from the bull.

The question of transmission of BLV infection by viral genome incorporated in ova from infected cows is being examined by BLV hybridization of concepti from ova transplanted from infected to noninfected cows. Similar transplanted ova are being followed to term and the calves tested for BLV antibodies. Thus far the calves have been serologically negative at birth.

Table II. Age in Years, When Progeny in Herds 1, 2, and 3 Became BLV Reactors (expressed in percent)

Dams	1/2-1	1-2	2-3	3-4	4+	Total
157 reactors	3	5	8	4	4	22
345 non-reactors	1	5	7	4	4	21

Table III. Age When 126 Cows With a Previous Negative Test Became BLV Reactors

Herd	1-2	2-3	3-4	4-5	5-6	6+
# 1	0	5	12	4	4	10
# 2	0	11	5	4	4	4
# 3	7	16	11	7	11	11
Total	7	32	28	15	19	25

Although early work had suggested that the high prevalence of BLV infection in certain cow families (30) was vertical transmission of virus, it was indicated in a later report that this was due to high rates of BLV infection in these herds (28).

BLV-infected cows probably shed virus in the milk either as virions or infected lymphocytes. Both sheep and newborn calves have been experimentally infected by oral administration of infective BLV material (31, 32). The likelihood of newborn calves becoming infected from colostrum was assumed and recommendations made to avoid this possibility. However, under natural herd conditions, calves born to BLV infected cows receive antibody in colostrum (which may neutralize BLV in the milk) and usually remain free of infection for one or more years (14). This evidence comes from three infected herds during seven years of testing (Table I). Data for herd #3 has been expanded to include four breeds of dairy cattle. The average annual infection rates in these herds were 27 to 63%. In calculating the data, positive responses in calves less than six months old in infected herds were disregarded because they could have been caused by colostral antibodies. The subsequent annual rate of reactors (21 to 22%) was the same in progeny from 157 infected dams as in calves from 345 non-infected dams (Table II). In these herds, most cows became reactors after they were two years old (Table III). Similarly, very low rates of BLV reactors in calves six to twelve months of age had been observed in otherwise highly infected herds studied in Canada (33) and Germany (34).

There is a possibility of lymphocytes passing through the placenta of a BLV infected cow thus causing prenatal infection of her calf. In some herds this might be due to an unrecognized break in the normal placental barrier. For example in the BF herd of Pennsylvania, where all the cattle over 30 months old have BLV antibodies, 18% of the newborn calves were found to have BLV antibodies before their first meal of colostrum indicating prenatal BLV infection (35).

Although antibodies develop within a few weeks after experimental exposure to BLV (32), serological evidence of natural infection usually occurs after cattle are one or more years old (18, 26, 33, 34, 35, 36 and Table III). This indicates a very slow spread of BLV.

Since intradermal inoculation of only 2,500 BLV infected lymphocytes (0.0005 ml blood or less) has established the infection in calves (32) the transfer of even small amounts of blood from BLV infected to susceptible cows must be avoided. Such inadvertent transmission could occur with needles and instruments.

With the introduction of serological testing for evidence of BLV infection, has come a new method for evaluating the extent of bovine leukosis. The following information was presented at the Third International Symposium on Bovine Leucosis held October, 1978 at Alfort, France (4).

Dr. Hoff-Jorgensen surveyed 587 Danish herds and forty-two of 372 hematologically suspect herds contained BLV

reactors.

Dr. Kaaden, Germany, tested one herd of 286 Friesan cattle every two weeks for four years to follow the spread of BLV infection. In this period the incidence of infection rose progressively from 4.8% to 52.6%.

In a high BLV incidence area of southwest France, Drs. Crespeau and Villaume found reactors in 55% of 271 herds tested in 1976. Of the 1,966 cattle surveyed, 25.4% were reactors. In 1977, 64% of 295 herds were positive. The incidence rate was 27.3% in the 2,214 cattle tested. The infection rate per herd ranged to 82%; a tumor rate of 100 per 100,000 was estimated.

Control of Bovine Leukosis by Serological Testing

The AGID test has also been applied in laboratories of European countries for control of BLV infection (4). Dr. Mammerick of Belgium tested 5 herds at 5 month intervals and reacotrs were killed within 1.5 to 2 months of each test. No additional reactors were found at the fourth and fifth test. In Germany, Dr. Schmidt tested 500 herds (20,000 cattle) in a high incidence area of Lower Saxony. During a two and one-half year period, the cattle were tested five times. After each test, the reactors were slaughtered. Using only this control program, the rate of BLV infection in many of the initially heavily infected herds had fallen to less than one percent. Dr. Straub, also in Germany, described results after removal of reactors more than six months of age from non-reactors. He tested several herds at three month intervals: On the first test, 21% of the cattle were reactors; after the second, 4.3% were reactors, and less than one percent at the third tests.

Forschner *et al* (37) has emphasized that the formerly accepted concept of vertical transmission of BLV infection must now be replaced by the idea that horizontal transmission is the more common means of natural infection. In a preprint of a report to the XIII Congress Deutsche Veterinarmedicine Gesellschaft and personal communication, Forschner provides much pertinent information from his government laboratory in Hannover. More than 250,000 AGID tests for BLV infection were done in the past two years. By removal of reactors about 90% of infected herds became free of BLV infection as indicated by subsequent tests. This was accomplished with removal of reactors after one test of a herd if there were only strong serological reactions and if there were no reactors in young (less than two year old) cattle. On farms where there were weak reactors or reactors among young cattle, subsequent tests often revealed more reactors which were then removed. The weak reactions were believed to occur with recent infection suggesting active spread of BLV. The above was true if there was no purchase of new animals. Forschner observed no familial association or evidence of vertical transmission. Variations in the strength of reactions (weak or partial bending of precipitin lines) were noted in tests on forty reactors in a 180 cow herd studied during eighteen months, though generally the strength of the precipitin line increased. In some of these cows, the AGID test was negative (probably very low antibody titer) for brief intervals and this could not be correlated with other conditions such as intercurrent disease, pregnancy or parturition. He recommends elimination of all reactors at the initial test, with a second test eight weeks after culling and subsequent tests at four month intervals, with removal of reactors, whether strong or weak, after each test. Forschner is emphatic that such tests must be done on the entire herd. Questionable individuals should be tested every two weeks until either clearly negative and retained, or eliminated if reactors. On this basis herds would be certified leukosis free.

In the U.S., a preliminary attempt at control of BLV infection by restriction of contact between infected and non-infected cattle indicated the feasibility of such a program (38). This was done in one dairy herd, one group of experimental cattle and one bull stud. The observations were limited to a little over a year. Management and purchase of new animals were problems in two of the groups, but indications were that control could be achieved.

Thus, it appears practical to eliminate BLV-infected animals when only a few reactors are in a herd; if there are many reactors, their replacements could be raised by separating then from the reactor cows after weaning. The degree of segregation needed is not known, but completely separate physical facilities for reactors and non-infected cattle may not be necessary, depending on housing and management of individual farms. Freedom from BLV infection could be established by raising and maintaining the young replacement non-infected (as measured by repeated serological tests) animals separately from that part of the herd with reactors.

BLV infection is usually subclinical and seems to have no other influence on health or milk production (39). Furthermore, since relatively few BLV infected cattle develop lymphosarcoma in the United States, the economic loss to dairymen is small.

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