Appendix

*Current Aspects of Bovine Leukemia

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Bovine leukemia (leukosis, lymphosarcoma) is a disease which has caused serious economic consequences in Europe. Apparently there are not as many herds in the United States that show as high a percentage of tumors as are found in European cattle. It is difficult to evaluate the actual incidence of the clinical disease in cattle in the United States. In 1974, 4,669 cattle carcasses were condemned because of lymphosarcoma (14). In general, we can assume that these cattle did not demonstrate clinical signs of lymphosarcoma before slaughter, but were condemned due to the presence of internal tumors since only five cattle were condemned on ante-mortem inspection. It is difficult to determine the number of cattle observed to have tumors and not sent to slaughter, but rather "condemned" by the veterinarian and sent for tankage from the farm.

Oncornaviruses, a name for oncogenic or tumorogenic RNA viruses, generally do not produce a high percentage of tumors in infected animals. They are insidious agents which can also cause abnormalities other than tumors. Among these is depression of the immune response with a concurrent increased susceptibility to other diseases. As well, infertility and decreased meat and milk production may occur. The true impact of bovine leukemia on production is very difficult to assess without a serological test to study the epidemiology of bovine leukemia (BL). Such a test could be used to diagnose BL and evaluate the production efficiency of infected herds in comparison with non-infected herds.

European countries are considering adaptation of a specific serological test to replace the lymphocyte count method of detecting animals with bovine leukemia.

We would like to describe the epidemiology of bovine leukemia, the experimental disease, and then a serological test which has been described recently by workers at the National Animal Disease Center. Data developed in the past year in our own laboratories will also be described.

Some years ago, Bendixen (2) concluded that persistent lymphocytosis was a precancerous stage of bovine leukemia. European countries used lymphocytosis as a means of detecting and attempting to eradicate bovine leukemia. As European eradication programs progressed, it was noted that many cattle developed tumors without developing persistent lymphocytosis. This indicated that animals could be selected which could not elicit a lymphocytosis in response to infection with bovine leukemia virus (BLV), but which were still susceptible to infection and the oncogenic potential of the virus; that is, they could still develop tumors. For this reason, many workers in Europe feel that BL cannot be eradicated solely on the basis of lymphocyte counts. We must consider persistent lymphocytosis as an indicator of infection with bovine leukemia virus, but evidently it is not a reliable sign of the disease.

Ferrer, et al. (4), have found BL to be a disease of low communicability both *in utero* and by contact or horizontal spread. They found about 10% of the fetuses of infected dams were infected at birth. The majority of young calves separated from positive dams remain negative until reintroduction into the herd at 19 to 24 months of age when they become infected apparently by contact with positive milking stock. Olson, et al. (11), have also found very few animals less than two years of age positive except for transient colostral antibody.

Naturally infected cattle provided the material for Miller, et al.(6), to first culture bovine leukemia virus in 1969 from lymphocyte cultures. Miller, et al. (10), inoculated 14 calves with lymphocyte cultures containing bovine leukemia virus that were prepared from cows that had died with lymphosarcoma and from two experimentally inoculated calves. All of the calves became infected with the BLV within 13 months as evidenced by its re-isolation. Five of the calves developed persistent lymphocytosis, which appeared between four and 13 months after inoculation.

Schmidt, et al. (13), have recently inoculated nine calves from herds negative for bovine leukemia with cell-free tissue culture fluid from lymphocyte cultures of a cow naturally infected with BLV. Nine other calves were inoculated with culture fluids from a cow not infected with the virus. None of the calves inoculated with the normal, or uninfected material, developed lymphocytosis or antibody to BLV, nor could virus be isolated from them. Three calves given

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the fluid from a naturally infected cow did develop lymphocytosis, antibody to BLV, and virus could be isolated from these three calves.

None of the calves in the two studies described were held long enough to develop tumors. However, the development of persistent lumphocytosis in eight calves is significant.

Olson, et al. (12), inoculated 13 lambs one to two weeks of age with lymphocytes from bovine lymphocyte cultures producing BLV particles. Eleven of the lambs became positively infected as shown by re-isolation of the bovine leukemia virus from their lymphocytes. Within 27½ months, five of the lambs had died from leukemia or lymphosarcoma.

These studies demonstrate that bovine leukemia virus 1) can be isolated from naturally infected cattle showing the disease, 2) can be used to infect calves in which it does produce a lymphocytosis, 3) does induce antibody formation in inoculated calves, and 4) can be re-isolated from inoculated calves. Although to this date no one has shown the oncogenic or tumor-producing potential of BLV in cattle, the inoculation of another ruminant, the lamb, did demonstrate that this virus can cause tumors in a ruminant animal.

One of the difficulties in studying the oncogenic potential of bovine leukemia virus in cattle is the expense of maintaining cattle for a long enough period of time to allow them to develop the tumors. We would expect that it would be necessary to hold the animals for five to seven years. This could also explain why the incidence of clinical disease in the United States is less than in Europe. On the average, our cattle are slaughtered by four to five years of age, whereas many animals in European herds are kept for 10 to 12 years, or longer.

Miller, et al. (6), cultured BLV in short term lymphocyte cultures from infected cattle, and later, Van Der Maaten and Miller (15) adapted the virus to grow in fetal lamb spleen cultures. This latter development allowed the production of viral antigens in large quantity for serological studies. The first antigen discovered was an ether-stable antigen called p24, or internal antigen of the virus. Numerous serological studies were done with this antigen and it was shown that infection with the virus correlated with the presence of antibody (1,3,5). Recently, a second antigen of the virus has been found which is sensitive to ether. It is a glycoprotein antigen, and was recently described at a bovine leukemia conference held in Copenhagen by Miller and Van Der Maaten (8). Our laboratories have been working with this glycoprotein antigen for approximately seven months and examining its use in the diagnosis of BL. We have correlated it with other serological tests.

Figure 1 shows an oncornavirus. On the inside is the p24 internal antigen which was the first one used for serological testing. The envelope of the virus contains the glycoprotein antigen. The immunodiffusion test which we have used employs the glycoprotein antigen. The test is similar in principle to the one used for equine infectious anemia. It is called the bovine leukemia-glycoprotein immunodiffusion test (BL-GID Test). We have compared the presence of antibody in cattle sera as determined by the glycoprotein antigen, the ether stable p24 antigen, and the complement fixation test.

Table 1 shows the relationship of antibody detected using the p24 antigen compared to the glycoprotein antigen. Both tests agreed that there were 22 animals positive and 17 animals negative. However, in 25 cases, the BL-GID test was positive while the p24 antigen test was negative. These represent 25 animals that were infected but undetected by the p24 antigen.

Table 2 demonstrates in more detail the relationship of these two tests. Both tests showed 22/64 animals positive; that is, 34%. However, the p24 test was negative and the BL-GID test was positive for 25/64; that is, 39% were missed by the p24 test. There were no animals positive on the p24 test that were negative on the BL-GID test, and both tests agreed that 27% were negative. The glycoprotein antigen BL-GID test was significantly ($P > 0.005$) more sensitive in detecting antibody to BLV than the immunodiffusion test employing the p24 antigen.

Miller and Van Der Maaten (7) developed a complement fixation test for antibodies to BLV antigens. They (8) compared the relative sensitivity of it and the ether stable p24 antigen, and found that the CF test was indeed much more sensitive in detecting infected animals. We compared the complement fixation test to the BL-GID test on samples that were tested at NADC with the CF test. These samples were tested on a blind basis using the BL-GID test. Forty-five animals were agreed to be positive by both tests, 16 were negative by both tests (Table 3). In one case, the CF test found antibody that was not detected by the BL-GID test. These studies showed an extremely significant agreement between the CF and the BL-GID tests ($P > 0.005$).

The commonly used lymphocyte count, the Bendixen Key, has been used in Europe and unofficially in the United States to determine animals infected with BLV. We studied 653 animals in 12 dairy herds. We only did one white cell count in contrast to the three that are normally accepted to verify that an animal is positive, so that we could have included animals which would be negative on the second or third count. Table 4 shows that 14% of the animals were positive on both tests upon the first count, and that 26% were negative on the BL-GID test, but positive on the Bendixen Key. With this one sample, these animals could have been considered false-positive reactors with the Bendixen Key. However, 19% were positive on the BL-GID test, but negative on the Bendixen Key. These animals would not have been retested based upon white cell count, but, indeed, were infected with bovine leukemia virus. This is an extremely high percentage of BLV infected animals to miss in a population, and shows the lack of sensitivity and specificity of the lymphocyte count method to determine animals infected with BLV.

FIGURE 1 ONCORNAVIRUS SCHEMATIC

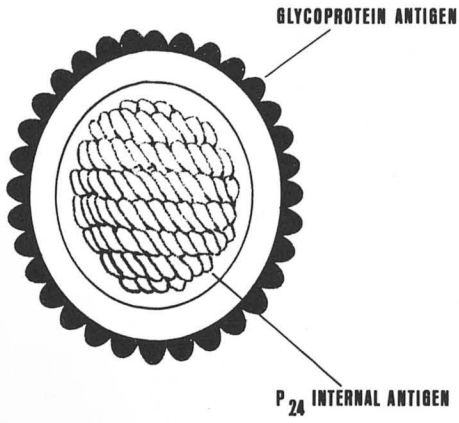

Table 1

Comparison of BL-GID and P 24 Antigen I.D. Test for Detecting Antibody to BLV

		BL-GID	
		+	-
P-24	+	22	0
Antigen	-	25	17

P > 0.005

Table 2

Animals with Antibody to BLV: BL-GID and P24 Antigen I.D. Tests

Positive P24, Positive BL-GID	22/64	34%
Negative P24, Positive BL-GID	25/64	39%
Positive P24, Negative BL-GID	0/64	0%
Negative Both	17/64	27%
Total		100%

P > 0.005

Table 3

Comparison of BL-GID and BL-CF Test for Detecting Antibody to BLV

		CF Test	
		+	-
BL-GID	+	45	0
Test	-	1	16

P > 0.005

Table 4

Comparison of Bendixen Key with BL-GID Test

Pos. BL-GID and Bendixen Key	94/653	14%
Neg. BL-GID, Pos. Bendixen Key	101/653	16%
Pos. BL-GID, Neg. Bendixen Key	125/653	19%
Negative Both	333/653	51%
Total		100%

P > 0.005

Table 5

Dairy Herd Survey for BL-GID Positive Animals

Herd No.	No. Pos./No. Tested	Percent Positive
1	0/50	0
2	1/44	2
3	9/65	14
4	21/88	24
5	20/74	27
6	20/62	32
7	21/55	38
8	29/42	69
9	29/40	72
10	47/64	73
11	30/39	77
12	24/28	86
	Total 251/651	39%

Range Positive = 0 to 86%
Median Positive Approx. = 32 to 38%
Weighted Mean Positive = 39%

Table 6

Beef Herd Survey for BL-GID Positive Animals

Herd No.	20 Cattle Tested	Percent Positive
1	0	0
2	0	0
3	0	0
4	1	5
5	1	5
6	2	10
7	3	15
8	4	20
9	4	20
10	5	25
11	8	40
12	9	45
13	9	45
14	14	70
15	18	90

Range = 0 to 90%
Median = 20%
Weighted Mean 78/300 = 26%

Both the tests were negative in 51% of these samples. There was a statistically significant ($P > 0.005$) difference in the specificity and sensitivity of the BL-GID test compared to the Bendixen Key methods for detecting animals infected with BLV.

Twelve dairy herds from Pennsylvania and New Jersey were screened for animals possessing antibody to bovine leukemia virus by the BL-GID test. A total of 651 animals were examined (Table 5). Various herds exhibited a range from 0 to 86% positive. The weighted mean positive animals was 39% (251/651 animals). The median herd incidence of infection was between 32 and 38%. In other words, based on this data, you would expect to find 32 to 38% of the animals infected in a randomly selected dairy herd.

Fifteen beef herds (14 from Mississippi, 1 from Texas) were examined for BL-GID positive animals. The range positive varied from 0 to 90% (Table 6). The weighted mean positive animals was 26%. However, the median percentage infection was only 20%. This means that if a herd was examined at random, you would expect to find 20% of the animals infected. These data imply a substantially lower infection rate in beef cattle than in dairy cattle.

In summary, bovine leukemia appears to be a disease that is not highly communicable; nevertheless, it is contagious in that it can spread horizontally, i.e., from one animal to another in a herd. The economic loss caused by bovine leukemia is difficult to evaluate but is considered substantially greater than that incurred by carcass condemnation. The virus of bovine leukemia causes a persistent lymphocytosis in some infected cattle. This has been related epidemiologically to a pre-cancerous state and persistent lymphocytosis has served as a basis for eradication programs in Europe. The lack of specificity of the lymphocyte count method was shown by the lack of correlation between it and the specific BL-GID serological test for detection of antibody to bovine leukemia viral antigen in 651 dairy animals. In that study, 19% of the cattle were infected, but were called negative by the lymphocyte count method. These animals could stay in the herds as unsuspected shedders of bovine leukemia virus and infect susceptible animals in contact with them.

The BL-GID test uses a viral glycoprotein antigen which is more likely to induce an antibody response in infected cattle than is the p24 antigen originally used to serologically study bovine leukemia. The test is comparable in sensitivity to the complement fixation test which has been shown to detect infection both earlier in the disease and in a higher percentage of animals. Indeed, it is felt that the complement fixation test is more sensitive than the p24 test due to the presence of the glycoprotein antigen in the antigen used for the CF test.

Our studies have shown that there appears to be more animals infected in dairy herds than in beef herds. This could very well be due to the management of the various herds. Dairy calves may have more contact with pooled colostrum, with nurse cows, and perhaps more intimate contact with infected animals than do beef calves who generally nurse their own dams and are kept a greater distance from other beef cows.

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