Relationship of the Antigens of Bovine Leukemia Virus to Clinical Enzootic Bovine Leukosis in Cattle

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Summary

Serum specimens from cattle which comprised 385 submissions over a 24-month period (1981-1983) were examined by the agar-gel immunodiffusion (AGID) test for the presence of antibodies to bovine leukemia virus (BLV). Of the specimens tested, 163 or 42% were positive by the AGID test for antibodies to BLV. Of the BLV-seropositive cattle, 34 or 21% were animals which had a diagnosis of enzootic bovine leukosis based on clinical signs (i.e., enlarged lymph nodes). Of the cases with clinical leukosis, 21 or 62% had antibodies only to the viral glycoprotein (gp) envelope antigen while 13 or 38% contained antibodies to both the gp antigen and the internal core viral antigen (p24). A clinical history was not available for 2 of the animals which had antibodies to both the gp and p24 viral antigens. In BLV-infected cattle, the titer of antibody to gp antigen was found to be consistently greater than to p24. However, the presence of antibody to p24 viral antigen in cattle appeared to be correlated with the clinical (tumorous) manifestation of leukosis. The detection of antibody to the p24 antigen of BLV in sera of cattle and its relationship to clinical (tumorous) enzootic bovine leukosis is discussed.

Introduction

Cattle infected with bovine leukemia virus (BLV), the etiologic agent of the adult (enzootic) form of bovine leukosis, develop antibodies to several viral proteins which include the envelope glycoprotein (gp) and a major internal core non-glycosylated protein (p24) (1, 2, 3, 4). The detection of antibodies to these viral proteins by serology provides the rapid method for the diagnosis of an infection by BLV in cattle (3,4,5). Previously, a diagnosis of bovine leukosis had been determined by the Bendixen test based on hematological keys and persistent lymphocytosis (6). The present serologic assays used for the detection of an infection by BLV include: agar-gel immunodiffusion (AGID), radioimmunoassay (RIA), enzyme-linked immunoassay (ELISA), complement-fixation (CF), and virus neutralization by the syncytial-inhibition test (3, 4, 7, 8, 9).

Of these preceding assays, the AGID test has been reported to be the least sensitive (5, 6, 9), but the AGID test is the most used assay for detection of BLV because of its ease, reproducibility, and economics. In a study of cattle in Oklahoma, the number of BLV-infected cattle was reported to be between 40 and 43% of those animals tested by the AGID and virus-neutralization tests (10). We previously reported (11) a 41% rate of infection by BLV in cattle in Oklahoma as detected by the AGID test.

In a summary of previous serologic surveys in the United States, the infection rate with BLV was reported to be 13 to 48% of the dairy and 14 to 19% of the beef cattle (6).

The eradication of BLV in herds with a history of lymphosarcoma has been accomplished by the detection of antibody to p24 antigen by the RIA test (7). In the preceding study (7), calves from leukemic mothers were tested twice within a 60-day period before their certification as free of infection with BLV. Eradication of BLV was reported in 90% of leukemic herds by use of the AGID test, but 2 tests were required within a 6-month period (6). However, in those herds tested for BLV by the AGID test, reactors have been found 2 to 3 years later (6). In eradication programs in Europe, 3 or more AGID tests have been done over a year period to identify all reactors (7).

Previously, we reported (11) on the detection of antibody to p24 by the AGID test in sera of 5 animals with clinical leukosis. Our data (11) confirmed similar findings (8) that the titer of antibody to gp antigen in cattle with clinical manifestations of leukosis (i.e., enlarged lymph nodes) exceeded that for p24 viral antigen.

Although the majority of serologic tests for the detection of BLV will distinguish between BLV-infected and non-infected cattle (2, 3, 4), none of these tests can predict clinical (tumorous) disease. Only the presence of clinical parameters as described by Stober (12) can presently be used to detect the small percent (0.02 to 5%) of cattle which eventually develop lymphosarcoma (6, 10).

Thus, the available serologic procedures have been used successfully in eradication programs for BLV (6, 7, 9). For veterinary practitioners, the diagnosis of clinical enzootic bovine leukosis (i.e., lymphosarcoma) and the culling of affected cattle would be enhanced if any of the present serologic parameters could be related to the eventual clinical (tumorous) disease in the animal.

The purpose of this report is to present data on the increased correlation of the clinical manifestations of leukosis in cattle with the p24 antibody detected in cattle sera by the AGID test.

Materials and Methods

Sera: Between July 1981 and July 1983, a total of 385 cattle serum samples from submissions to the Oklahoma Animal Disease Diagnostic Laboratory (OADDL) were tested for antibody to BLV by the AGID test.

Clinical History of Cattle with antibody to gp and p24 viral antigens:.

Case History No. 1: A 10-year old female Holstein was noticed recumbent, found to be anemic and had enlarged external lymph nodes.

Case History No. 2: A 6-year old female Holstein from a herd of 200 was found recumbent with posterior paralysis. No enlarged lymph nodes were observed.

Case History No. 3: A 7-year old female (breed unknown) was examined and found to have swellings along the back and also a history of respiratory distress.

Case History No. 4: A serum sample from a 6-year old female (breed unknown) was submitted for a BLV test, however, no further history was available.

Case History No. 5: A 4-year old female Angus from a herd of 35 which had a history of lymphosarcoma, was found to have enlarged supramammary lymph nodes and enlarged lymph nodes on rectal palpation. Following the death of the cow, an examination of the abomasum revealed a tumor mass.

Case History No. 6: An 8-year old female Angus/Holstein cross had been losing weight and not eating well for three weeks. The animal was later described to be recumbent and a multinodular mass was subsequently detected in the abomasum following death.

Case History No. 7: A 5-year old female Santa Gertrudis which had calved 6 months earlier had been in an emaciated state, however, no further history was given.

Case History No. 8: A serum sample from an adult female Holstein was submitted for a BLV test. No additional history was given.

Case History No. 9: A 4-year old male Angus had a clinical history of posterior paralysis of the hind-quarters.

Case History No. 10: A 6-year old female Holstein had enlarged left prefemoral lymph nodes and enlarged supramammary lymph nodes; however, clinical pathology indicated the lymphocytes were normal.

Case History No. 11: A 3-year old female Holstein was observed to be depressed and anorectic. Upon examination by palpation, the lymph nodes were found enlarged and abdominal masses were detected after a rectal examination.

Case History No. 12: A 9-year old female Santa Gertrudis was observed to have a heavy-doughy uterus with a possible tumor. The cow was housed in a herd which had a 50% conception rate. Other cattle in the herd had been found to contain enlarged prefemoral lymph nodes. Of the cows in the herd which were tested by the AGID test, 2 animals had antibodies to only the gp antigen of BLV.

Case History No. 13: An 8-year old female Angus had a gradual weight loss with ventral edema and, by palpation, the superficial lymph nodes were greatly enlarged.

Case History No. 14: A 4-year old female Holstein was recumbent and no enlarged lymph nodes were found upon examination. The cow was from a herd of 80 which had a previous history of infection with BLV. From the herd, serum samples from 10 randomly selected animals were tested by the AGID test for antibodies to BLV (Table 1); 8 cows were seropositive for the gp viral envelope protein of BLV. Of these sera, 10 were tested in a serologic profile for antibodies to several viral agents (Table 1). The antibody responses against the viral agents tested were unremarkable. Athough the herd had a nutritionally adequate diet, the cows within the herd had a non-responsive milk fever during lactation. In the last 2 years, approximately 7 cows have been found with enlarged lymph nodes upon palpation.

Case History No. 15: A 1-year old female Angus died of bloat and, at necropsy, malignant tumors were found at the base of the heart and in mesenteric lymph nodes. The tumors were diagnosed as lymphosarcomas.

Procedure for the Agar-Gel Immunodiffusion (AGID) Test:

The AGID test (4) for the detection of antibodies to BLV was performed as previously described (11) with commercial reagents.[a] Following an initial screening, cattle sera which produced more than 1 line of identity with reference serum against the commercial reference antigens (gp51 and p24) were subsequently tested with "enhancement" serum[b] which contained antibodies to gp and p24 viral antigens. To determine the concentration of antibodies to p24 antigen in the serum from each animal, two-fold dilutions of each serum were made and tested in the AGID test. Each serum was then tested against commercial antigens using either reference or "enhancement" serum.

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[a] Pitman-Moore, Washington Crossing, New Jersey 08560

[b] Reference p24 antiserum was kindly provided by Dr. J. M. Miller, National Veterinary Services Laboratory, and Dr. L. D. Miller, Iowa State University, Ames, Iowa 50010

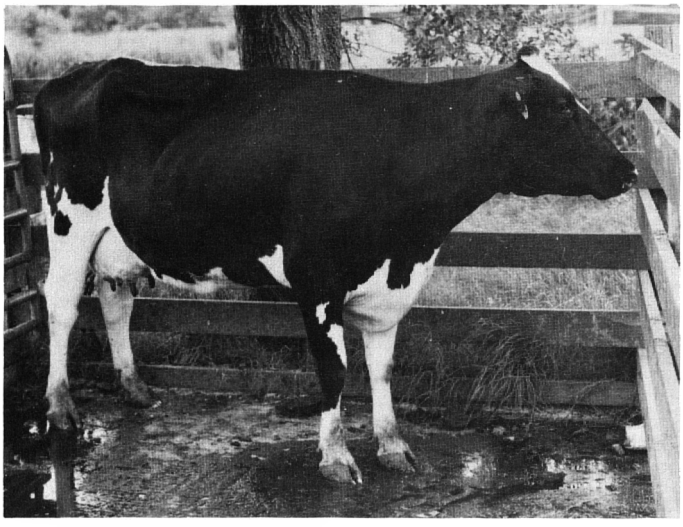
TABLE 1. A Serologic[1] Profile of Cattle From a BLV-Infected Herd

Animal No.	Viruses Tested				
	BTV	BLV	BVD	IBR*	PI_3*
1	+	+	0	16	5
2	0	+	0	4	10
3	+	+	+	<4	40
4	+	0	+	24	5
5	+	+	+	<4	20
6	+	+	+	48	10
7	+	0	+	48	5
8	+	+	0	24	20
9	+	+	+	4	10
10	+	+	+	<4	10

[1]Antibodies to bluetongue virus (BTV) and BLV were determined by AGID with serum which contained antibodies to gp51; to bovine viral diarrhea (BVD) virus by an indirect immunofluorescence test; to infectious bovine rhinotracheitis (IBR) by the serum-neutralization test; and to parainfluenza type 3 (PI_3) virus by the hemagglutination-inhibition assay.

*Antibody Titer
+ = Positive, 0 = Negative

Figure 1. A clinically normal 4-year old Holstein cow from a herd infected with BLV (Case No. 14, Table 2) which had antibodies to gp and p24 viral antigens.



Results

The clinical history in most submissions from affected cattle included: enlarged lymph nodes, paralysis, recumbency, respiratory distress, palpable tumors and enlarged masses. The most frequently described clinical sign was an enlargement of the lymph nodes. Cattle with these clinical histories were designated as clinical cases of enzootic bovine leukosis. In case no. 14, a Holstein cow (Figure 1) in a leukemic herd (Table 1) was found to have periodic episodes in which it was recumbent and anorectic but no enlarged lymph nodes were present. A malformation of the udder was not seen and the mammary lymph nodes in this cow were not enlarged (Figure 2).

Of the 385 cattle sera submitted to OADDL for testing for antibodies to BLV, 163 or 42% of the specimens were seropositive for antibodies to the viral envelope glycoprotein (gp). Thirty-four or 21% of these seropositive animals were diagnosed as having the clinical signs of enzootic bovine leukosis. However, 21 or 62% of these clinically-ill animals were found to contain antibodies only to gp viral antigen. Of the remaining 13 animals, all had antibodies to both gp and p24 viral antigens. Sera from 2 animals had antibody to both gp51 and p24 viral antigens but no clinical history was available (Table 2). The age of the clinically-ill cattle which had both types of antibodies ranged from 1 to 10 years with an approximately equal distribution between dairy and beef breeds.

Figure 2. Palpation of the udder of the cow (Case No. 14, Table 2) which had no abnormalities or enlargement of the mammary lymph nodes.

In each of 13 sera which were found positive to p24 and gp by the AGID test, the level of antibody to p24 viral antigens ranged between 4 to 128 (Table 2). Antibody titers to gp viral antigen found in most sera were consistently higher than those to p24. The amount of antibody to p24 viral antigen present did not appear to be related to the severity of the clinical signs observed in the animal. In a comparison of the commercial reference serum in the AGID test to a reference "enhancement" serum with known antibodies to gp and p24

TABLE 2. Summary of the Clinical History of Cattle with Antibody to gp and p24 Viral Antigens

Case History No.	Breed	Sex	Age (years)	Antibody Titers[a] to p24 Antigen	Clinical History Reported
1	Holstein	F	10	8	Enlarged lymph nodes
2	Holstein	F	6	ND*	Posterior paralysis
3	Unknown	F	7	4	Swellings on back
4	Holstein	F	6	64	None described
5	Angus	F	4	64	Enlarged lymph nodes; BLV-herd
6	Angus/Holstein	M	8	16	
7	Santa Gertrudis	F	5	128	Mass in abomasum
8	Holstein	F	Adult	4	Emaciation
9	Angus	F	4	128	None described
10	Holstein	F	6	16	Posterior paralysis
11	Holstein	F	3	16	Enlarged lymph nodes
12	Santa Gertrudis	F	9	ND	Enlarged lymph nodes
13	Angus	F	8	32	Enlarged lymph nodes
14	Holstein	F	4	32/16**	Recumbent; BLV-herd
15	Angus	F	1	16	Tumor internally

*ND = not done ** = Acute/convalescent serum titer
[a]Antibody titers to gp51 were greater than 128

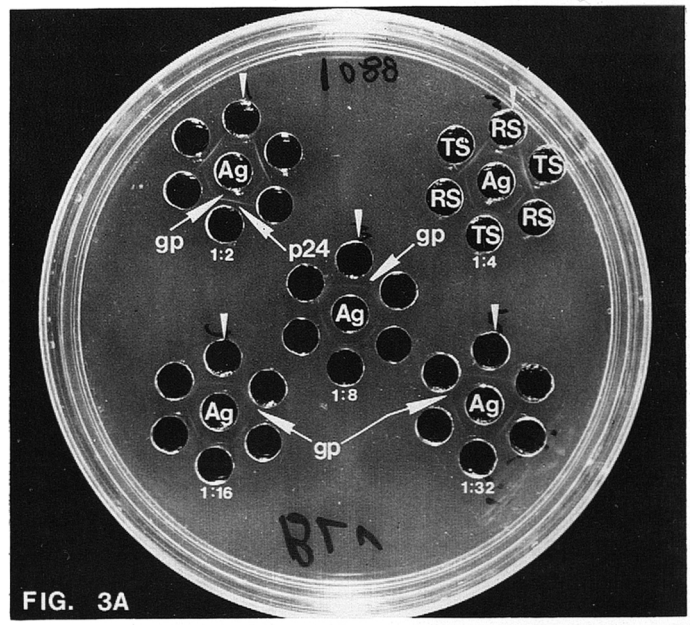
viral antigens, the precipitin line of identity to p24 viral antigen produced with each seropositive cattle serum was readily visible with the "enhancement" serum but not the commercial serum (Figure 3). However, both reference sera did produce visible lines to the gp viral protein in each of the seropositive sera tested.

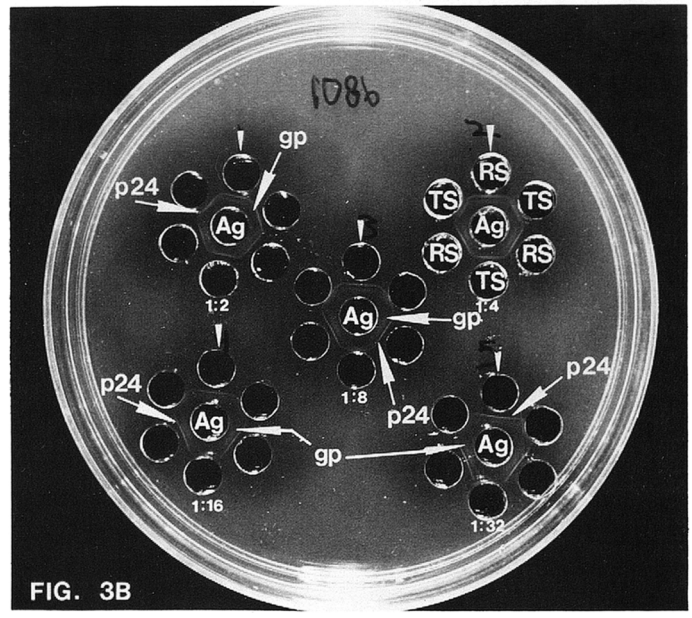
Discussion

Enzootic bovine leukosis has been described clinically as an enlargement of superficial lymph nodes with exophthalmos of the eyes, lymphadenopathy, anorexia, lameness, paralysis and emaciation (12, 13). In reviews of the clinical parameters of bovine leukemia, 4 different forms have been described, namely: a) enzootic or adult, b) juvenile, c) thymic and d) cutaneous (6, 12). Only the enzootic form of bovine leukosis has been associated with bovine leukemia virus (2, 6, 13).

The incidence of clinical lymphosarcoma in herds infected with BLV in a midwest United States survey was estimated at 3% of cattle seropositive for BLV (13). The milk breeds of cattle, Holstein, Jersey, Milking Shorthorn, Guernsey and

Figure 3. Comparison of precipitin lines formed to gp51 and p24 viral antigens in the AGID test. Lines of identity with commercial serum (arrows). (B) Lines of identity with "enhancement" serum (arrows). RS = reference serum, TS = cattle serum, gp = viral glycoprotein, p24 internal core viral protein. Arrowheads indicate start of the clockwise alternate addition of RS and TS.





Brown Swiss have been reported to have a higher incidence of reactors for BLV than beef breeds (14, 15). In 1978, the economic losses in cattle to bovine leukosis was estimated to be $7,000,000 in the United States (14). The frequency of infection by BLV in the domestic cattle population in Oklahoma was estimated at 28% (10). The incidence of infection with BLV in the United States has been estimated as 0.02% in dairy cattle and probably lower in beef cattle (10). Other estimates indicate that there were approximately

18,000 cases of bovine leukosis caused by BLV in the United States in 1981 (6). Clinical lymphosarcoma is a rare occurrence. Nevertheless, serologic surveys indicate in those isolated herds that have BLV-infected animals, a large number may develop clinical (tumorous) disease (2).

In order to eradicate BLV from affected herds, various diagnostic tests have been employed. The Bendixen hematologic keys have been used as an indicator of persistent lymphocytosis. However, persistent lymphocytosis in cattle does not occur as a sequelae to infection by BLV and most cattle infected with BLV do not develop lymphosarcoma (16). Other investigators (17) have also reported that persistent lymphocytosis is not a reliable criterion of impending tumor formation.

The European Economic Community has adopted the AGID test as the only approved test to use for screening of cattle for BLV infection prior to importation of cattle and frozen semen into Europe (18). Several countries still permit a hematologic examination to qualify cattle for export, but requests for serologic tests for BLV are increasing (17).

Most serologic tests for BLV are more sensitive than the AGID test (8, 9, 10, 17); however, the AGID test has been adopted for general use because of low cost, ease of the technique, availability of reagents and reproducibility between laboratories (17). Currently, commercial reagents are available which measure the envelope glycoprotein (gp51) of the virus (19). Other investigators (8) have reported that the AGID test is subjective and does not detect all sera that have low level antibodies to BLV. Furthermore, although the AGID test detects antibody to BLV, it was not recommended as a test for the confirmation of tumorous leukosis (17). In a previous study, we found 41% of the cattle sera tested by the AGID test were positive for antibodies to BLV (11). Within this seropositive sample, 5 sera had antibody to both p24 and gp viral antigens and clinical leukosis was diagnosed in each animal as indicated by enlarged lymph nodes. In the present study, we have identified 34 animals with clinical histories of enzootic bovine leukosis of which 13 had antibodies to both gp and p24 antigens. However, a correlation between the severity of clinical signs and the titer of p24 antibody was not apparent. The level of antibody to gp antigen in these clinically-ill animals was higher than antibody to p24 viral antigens. Lines of identity for antibodies to p24 viral antigens were visibly stronger by use of an "enhancement" serum which contained antibodies to both p24 and gp viral antigens when compared to a commercial serum. Of the 15 animals with antibodies to p24, 13 had a history compatible to that described for clinical leukosis which suggests that the presence of antibody to p24 viral antigen in an animal may be a diagnostic indicator of tumor development.

Previous studies with the RIA and AGID tests with the p24 viral antigen indicate that the p24 viral protein can detect animals infected with BLV (7,8); but a correlation with clinical leukosis has yet to be established. In 2 separate serologic surveys conducted over a 4-year period, we have observed that the presence of antibody to p24 viral antigen in cattle sera is usually detected when animals have clinical manifestations of enzootic bovine leukosis.

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